Development and Evaluation of the Efficacy of a Local Probiotic in Comparison with a Commercial Probiotic in the African Catfish, *Clarias gariepinus*

Francisca George, Akinwale Akinleye, Adeoluwa Akinyemi
Department of Aquaculture and Fisheries Management
Federal University of Agriculture
Abeokuta Nigeria
georgefoa@funaab.edu.ng

Oluwatoyin Afolabi
Department of Microbiology
Federal University of Agriculture
Abeokuta, Nigeria

***Abstract*—Probiotics are mono or mixed cultures of live organisms which improve the host's health status and indigenous flora properties. The use of probiotics in enhancing growth and as replacement for synthetic growth promoters in animal health management and nutrition has been scientifically proven. This study was carried out to evaluate the intestinal flora of the African catfish, *Clarias gariepinus* for probiotic properties, develop suitable probiotic formulation/s from them; and evaluate the efficacy of the developed probiotic for promoting growth and health in the African catfish in comparison with a commercial probiotic, Mito yeast. Laboratory screening of the gut Microflora of cultured *C. gariepinus* using standard methods revealed *Lactobacillus plantarum* as a viable organism with probiotic properties. *Lactobacillus plantarum* (Accession no: KP 410238) was thereafter cultured, processed and included as supplement in the diet of *C. gariepinus* and evaluated for growth and health enhancement capability. Experimental diets (TD1, TD2, TD3, TD4) were supplemented with the developed probiotic containing *L. plantarum* (10^9 CFU/g) at 0, 0.25, 0.5 and 1% respectively, while TD5 was supplemented with 0.5% MY-500 (MitoYeast) containing *Saccharomyces boulardii* culture ($2x10^9$ CFU/g). Growth performance, measured by body weight gain, BWG; specific growth rate, SGR and metabolic growth rate, MGR; and nutrient utilization (protein efficiency ratio (PER), feed conversion ratio (FCR) and feed efficiency, FE) were significantly different ($P \leq 0.05$) in treatments with varied levels of probiotics' inclusion compared to the control diet. Hematological effects as indicated by Packed Cell Volume, Hemoglobin, Red Blood Cell count, White Blood Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration and Platelet count showed an increase in fish fed probiotic-supplemented diet treatments compared to control, but the effects were not significantly different ($P \geq 0.05$), and values were within recommended levels. This suggests that the developed probiotic has no negative impact on the health status of *C. gariepinus*. This study concludes that dietary inclusion of probiotics in *C. gariepinus* diets improved growth and nutrient utilization in the fish; and had no adverse effect on its health status. *Lactobacillus plantarum*, a naturally occurring microorganism present in the gut of *C. gariepinus* was identified and confirmed as a viable probiotic agent in the species.**

Keywords*—Probiotics, fish nutrition, Growth promoter, L. plantarum, C.gariepinus)

I. INTRODUCTION

This Aquaculture production have recorded a rapid growth rate of 8.9% per annum, compared to captured fishery and livestock production that grows at about 1.2 and 2.8% respectively as recorded by FAO in 2010. This appreciable increase is due to intensification of the cultured systems, continuous research and development in aquaculture systems.

In Nigeria, aquaculture production is drastically increasing and gradually becoming a means of livelihood to average farmers because of its continuous demand by the populace, readily available market and quick economic turn over. Presently, many small holding fish farmers and those on commercial production are exploiting these merits and suitable conditions towards better production [1].

Probiotics can be described as cultured products or live microbial feed supplement, which affects its host positively by improving intestinal balance and health status of the host [2]. Probiotics can further be described as pure cultures of one or more microbes included in feed that proliferate in the hosts gut, ensure balanced and beneficial microbial population and maintain a healthy status of the host animal [3]. Due to increase in research focusing on environmentally-friendly production, there is increasing aim at exploring the possibility of probiotics effects on aquatic organisms. Most of these focused mainly on improved health aspects with little focus on its nutritional benefit [4].

Recently, there are more focus on the research centered on use of probiotics in aquaculture [5]. As a result of its beneficial impacts, probiotics are now been used in aquaculture industry as a means to control diseases, improve on water quality and increase the immune system of its host [6]. Many of the strains in use belong to lactic acid bacteria (LAB), although many other microbes belonging to other species have also been tested [7].

Catfish (*Clarias gariepinus)* also called African sharp-tooth catfish belongs to the family Clariidae and are found throughout Africa and part of Middle East. Their habitat includes freshwater lakes, swampy areas, rivers, and artificial habitats like oxidation ponds, earthen pond etc. [8]. Catfish is a

preferred choice in the tropics because it grows fast, has ability to feed on vast agricultural by-products, hardy, tolerance to harsh water quality situations and it is more economically rewarding than tilapia as it can be sold live at the market. Based on the aforementioned qualities, catfish has become specie of choice among fish farmers in Nigeria and Sub-Sahara Africa [8].

There is a need to evaluate the nutritional importance of probiotic inclusion in aquaculture production and development of a probiotic strains from commonly cultured fish species to enhance growth and immune system of cultured species in Nigeria [9]. This research therefore, is aiming to focus on exploring the nutritional benefit of probiotics in the tropics, using Catfish (*Clarias gariepinus*) as experimental animal. It is thus expected that a positive result from the research will help in solving issues relating to high nutritional costs in aquaculture and helps in encouraging farmers towards environmentally-friendly aquaculture production in sub-tropics and tropical aquaculture.

II. Materials and Methods

A. Location

The feeding trial was sited at the Hatchery unit of Aquaculture and Fisheries Management Department, Federal University of Agriculture, Abeokuta, Ogun State, South-West Nigeria with coordinate 07°13'51"N, 03°26'18"E, while the laboratory experiments were conducted at the Microbiology and Biotechnology laboratory of the institution. Further screening and analysis as regards probiotics development was partly done at the Tuberculosis Laboratory section of Sacred Heart Hospital, Lantoro in Abeokuta.

B. Experimental Animal

A total number of 150 healthy catfish (*Clarias gariepinus*) juvenile with an average body weight of 15±0.53g were obtained from fish hatchery unit of Odeda farm Institute, Odeda, Ogun State and transported in an open 50 litres capacity plastic tank to the experiment site where it was emptied into a 200 litres plastic tank for acclimatization.

C. Experimental Design

Selected healthy African catfish, *Clarias gariepinus*, juveniles with average weight of 15.14± 0.35g were randomly distributed at the rate of 10 fish per tank into each of the 15 experimental tanks, there are 5 treatment in triplicate. The fish were acclimatized for 14 days and fed with control diet before data collection. The fish were fed twice daily, around 9.00am and 15.00pm daily at 3% body weight. The ration was adjusted weekly with reference to new mean weights measured. There was no observed leftover feed. The fish were not fed until after the weighing and when a new feed calculation is made in other to adjust the quantity fed for each feed in correspondence to their body weight. The experiment was performed for 12 weeks after which growth parameters and nutrient utilization was accessed.

D. Experimental Diet

A total of five isonitrogenous and isoenergetic experimental diets with crude protein levels of 40% and a calculated digestible energy level of 12kJ/g^{-1} according to (ADCP, 1983) were formulated. Probiotics was not included in the first diet and served as the control, while the remaining four diets were supplemented with cultured and developed probiotics containing *Lactobacillus plantarum* at 0.25%, 0.5%and 1% level of inclusion for diets DP1, DP2 and DP3 respectively while diet DP4 was formulated with inclusion of MY-500 (MitoYeast) containing Saccharomyces *boulardii* culture ($2x10^9$ CFU/g) at 0.5% to serve as a comparative diet to the first feeding trial.

The ingredients were measured using a sensitive scale and thoroughly mixed together by hand starting with minute ingredients to ensure proper homogenization of the ingredients. The diets were then pelletized using a 2-mm pellet press after which it was sun-dried and kept in airtight containers.

E. Probiotics Sample Preparation

10 table-sized Catfish (*Clarias gariepinus*) were each selected from university reservoir dam and Concrete tank pond of Federal University of Agriculture, Abeokuta, Nigeria. Collected samples were killed and the gut content was removed and kept in test tube. This was later homogenized and preserved for further analysis.

F. Screening of Bacteria for Probiotic Attributes

a. Tolerance to Low pH

Isolates was grown on MRS broth (LabM) at 30°C overnight and later sub-cultured into a freshly prepared MRS broth and incubated for a day. The cultures were then centrifuged at 5000 x g for 10 min. at 4°C. The pellets was then washed in sterile phosphate-buffered saline (PBS) (Oxoid), pH 7 and re-suspended in PBS. Each strain was diluted at 1/100 in PBS at pH 2, 3 and 4. Incubation time were observed at 2, 4 and 6 h. Bacteria was later transferred to MRS broth and incubated at 37°C overnight.

b. Bile Resistance

The isolates that were able to resist the adverse pH conditions were selected for bile tolerance determination. Growth was then accessed spectro-photometrically (600 nm) in MRS containing 0.3% bile salts (Sigma and catalogue no. B8381) and compared to bile salt-free MRS. Growth delay was later used to determine bile tolerance [10].

c. Antibacterial Sensitivity

Agar well diffusion method was carried out according to a modified method of [11]. LAB was grown in MRS broth for 24hours at 37°C, afterward centrifuged at 5340Xg for 15minutes to separate the cells from the supernatant which contained the antibacterial substance. Afterward, the supernatant was filter-sterilized using a Membrane Filter, collected and stored in sterile bottles. Nutrient agar was then prepared and allowed to cool to around 45°C and seeded with test pathogenic organisms, and poured into plates and allowed to set. Five wells was then bored into the gar with the aids of cock borer of 5mm diameter, and filled with 80microlitres of the filtered supernatant. Positive and negative control wells were filled with sterile MRS broth and standard antibiotics solution of 100microlitre/ml. This was afterward incubated for 24hours at 37°C. Zones of inhibition were then measured using

a vernier caliper, and any zone not up to 11 mm was counted as insignificant.

d. Resistance to Antibiotics

This was determined with the use of 10 different antibiotic discs (bioMérieux, Marcy-l'Etoile, France) on MRS medium. *Staphylococcus aureus* and *Enterococcus feacalis* were used as the control.

e. Haemolytic Activity of the Isolates

Freshly prepared lactobacilli broth cultures was splashed onto Blood agar plates that contains 50 ml of blood (Oxoid, Milan, Italy), and were incubated for 48 h at 30°C. The plates were then examined for α, β and γ-haemolysis signs.

III. Data analysis

The following Parameters were analyzed:

Growth Parameters and energy budget - Body weight, Body Weight gain (%) and Specific Growth Rate (SGR), Metabolic growth rate (MGR),

Nutrient Utilization - Feed conversion ratio (FCR), Protein efficiency ratio (PER)

The following formula were used for evaluation of growth parameters and nutrient utilization

BMG = Final body mass - initial body mass, BMG (%) = [(Final body mass - Initial body mass) / Initial body mass] * 100. SGR %= [(ln final body mass in g) - ln initial body mass in g) / number of trial days] * 100

MGR = (Body mass gain, g) / [{(initial body mass, g / 1000) $^{\wedge 0.8}$ + (final body mass,g / 1000)$^{\wedge 0.8}$}/2] / duration of the trial days (Dabrowski *et al*. 1986). FCR = dry feed fed (g)/body mass gain (g) PER = fresh body mass gain (g)/crude protein fed (g).

IV. Statistical analysis

Data collected were subjected to one-way analysis of variance (ANOVA) and the significance of the differences between means was tested by Duncan's multiple range test ($P<0.05$). SAS Version 9.1 (Statsoft Inc., Tulsa, USA) was used to determine the level of significance and values of expressions as means ± standard deviation.

V. Results

A. Growth and Nutrient Utilization

The result of growth performance and nutrient utilization in *C.gariepinus* juvenile fed diets with inclusion of locally developed and commercial probiotics after 12 weeks culture period is presented in table 2. There was a significant difference ($P \leq 0.05$) with regards to body weight gain (BWG), specific growth rate (SGR), metabolic growth rate (MGR) in treatments where probiotics are included when compared with control diet. Nutrient utilization was also significantly different ($P \leq 0.05$) with regards to protein efficiency ratio (PER), feed conversion ratio (FCR) and feed efficiency (FE).

B. Haematological Parameters

The haematological parameters recorded after at the end of 12 weeks culture period are presented in table 3. Significantly ($P \leq 0.05$), differences were observed in the haematological parameters measured between control diet and probiotic based diets. The result indicates a higher value in packed cell volume (PCV), haemoglobin (HB), red blood Cell (RBC) white cell volume (WBC); mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet (PLT) when diets where probiotics is included is compared with control diet at the end of the feeding experiment.

VI. Discussion

Probiotics are referred to as life microorganisms which once ingested in appropriate quantity, enhance its host's health status [12]. Probiotic are further regarded as living microorganisms that enhance and promote health status of their hosts by ensuring intestinal micro flora balance for optimal metabolic performance [13]. Recent research affirmed that probiotics inclusion in aquaculture could positively aid fish growth and cultured environments (bio-control and bio-remediation) of cultured aquatic fish [14, 15, 9].

This study reported a better significant growth performance in all diets supplemented with probiotics and this is similar to Al-Dohail *et al* [15] who stated that growth in Catfish diet supplemented with probiotics of *Lactobacillus acidophilus* was significantly ($P<0.05$) higher than control diet that was not supplemented. This also conform with Carnevali *et al* [14] who confirmed that sea bass juvenile growth was significantly enhanced in treated groups than the control diet with the use of *Lactobacillus delbrueckii* as a probiotic through rotifer carriers and Artemia nauplii after 70 days. This indicated an improvement in growth performance of fish and its healthy status despite the differences in the methods of inclusion and species used.

It is also worthy to note that the improvement in growth may be related to an enhancement of the microbial flora in the intestine as reported by [13] where a significantly higher PER was reported when fish was maintained on probiotics supplements than in control group. This result was also similar to Lara-Flores *et al*. [16] where it was reported that *Oreochromis niloticus* recorded better survival rate, SGR, PER and FCR parameters in treatments with *L. acidophilus and S. faecium*. This report in probiotic-treated group could be as a result of their increased potential to tolerate harmful conditions that fish may be exposed to in the culture tanks as affirmed by Rollo *et al* [17] in *Sparus aurata* (Sea bam).

The similarity in the previous studies and this present one showed a higher growth performance and feed efficiency with the use of probiotic (either commercial or developed). This could be attributed to higher nutrient digestibility, better absorption and enhanced enzyme activities which could be

TABLE I: Growth Performance and Nutrient Utilization in Clarias Gariepinus Juveniles Fed the Developed Probiotics Experimental Diets for 12 Weeks.

	Control	DP1	DP2	DP3	DP4
IBW (g)	15.10 ± 0.07	14.99 ± 0.03	15.06 ± 0.16	15.17 ± 0.14	15.12 ± 0.17
FBW (g)	43.39 ± 1.32^{b}	44.24± 2.0^{ab}	47.78 ± 1.33^{a}	52.77 ±3.99 ^{a}	50.61 ± 1.12^{ab}
BWG (%)	187.29 ± 9.86^{b}	195.18 ± 14.33^{ab}	217.30 ± 3.91^{a}	247.90 ± 14.5^{a}	234.78 ± 4.31^{a}
SGR (%)	1.26 ± 0.2	1.29 ± 0.6	1.37 ± 0.2	1.48 ± 0.1	1.44 ± 0.5
MGR (g/kg$^{0.8}$/day)	0.34 ± 0.01	0.35 ± 0.01	0.39 ± 0.01	0.45 ± 0.01	0.42 ± 0.02
PER	7.83 ± 1.17^{b}	7.98 ± 0.23^{ab}	7.93 ± 0.33^{ab}	9.38 ± 0.04^{a}	9.73 ± 0.43^{a}
FCR	0.32 ± 0.01	0.31± 0.02	0.30 ± 0.02	0.27 ± 0.01	0.26 ± 0.02
FE	313.15 ± 6.38	319.25 ± 17.27	319.13 ± 13.2	375.35 ± 1.54	389.37 ± 14.33

NB: IBW, Initial body weight; FBW, Final body weight; BWG, Body weight gain; SGR, Specific growth rate; MGR, Metabolic growth rate; PER, Protein efficiency ratio, FCR, Feed conversion ratio; FE, Feed efficiency. Values are mean (n = 3) ± standard deviation. For all parameters, mean values in the same column were not significantly different ($p < 0.05$).

Table 3: Developed Probiotic Diet Influence on Haematological Parameter of C. Gariepinus

	Control	DP1	DP2	DP3	DP4
PVC (%)	14.10 ± 0.02^{a}	14.21 ± 0.03^{a}	14.57 ± 0.02^{a}	17.39 ± 0.03^{b}	15.43 ± 0.02^{ab}
HB (gm/100)	2.73± 0.03^{a}	3.11 ± 0.02^{a}	3.48 ± 0.03^{ab}	5.91 ± 0.03^{b}	4.23 ± 0.01^{ab}
RBC (x10/ml)	1.53 ± 0.02^{a}	2.06 ± 0.02^{a}	2.17± 0.03^{ab}	2.52 ± 0.02^{b}	2.22 ± 0.03^{ab}
WBC (x10/ml)	19.13 ± 0.12^{a}	21.3 ± 0.02^{a}	24.71 ± 0.02^{ab}	31.07 ± 0.02^{b}	27.23 ± 0.04^{ab}
MCV (fl)	80.72 ± 0.03^{a}	83.51 ± 0.02^{a}	91.14 ± 0.01^{ab}	103.21 ± 0.04^{b}	92.28 ± 0.03^{ab}
MCH (gm/100)	34.31 ± 0.22^{a}	36.14 ± 0.15^{a}	37.27± 0.19^{ab}	38.41 ± 0.17^{b}	37.66 ± 0.14^{ab}
MCHC (gm/100)	40.07 ± 0.03^{a}	41.02 ± 0.03^{a}	41.72 ± 0.02^{a}	44.51 ± 0.03^{b}	43.09 ± 0.01^{ab}
PLT (x10/μl)	131.73 ± 0.04^{a}	136.41 ± 0..03^{a}	142.19 ± 0.03^{ab}	178.23 ± 0.02^{b}	163.07 ± 0.02^{ab}

NB: PCV, Packed Cell Volume; HB, Haemoglobin; RBC, Red Blood Cell; WBC, White Cell Volume; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; PLT, Platelet

attributed to proper intestinal micro flora balance as reported by [13], or exo-enzyme secretion as reported by Moriarty [18]. Comparably, Tovar-Ramirez [19], Wang *et al* [20] and Suzer *et al* [7] reported that digestive enzyme activities are better enhanced when fish was fed with diets where probiotics were supplemented. Furthermore, improvement in growth performance and nutrient utilization could as a result of lower stressor levels in fish fed with probiotics enhanced diets. Carnevali *et al*. [14] reported that when fish was fed a diet supplemented with *L. delbrueckii*, there was a decrease in cortisol levels which affects the transcription of insulin-like growth factor (IGF-1) and myostatin (MSTN), which are both known to be a regulator of growth performance and in effect leads to an appreciable increase in body weight of the fish when compared with the control diet.

There was no significant difference in water quality parameters observed between the fish that fed on probiotics

supplemented diets when compared with the control. This conform with the findings of Wang and Zirong [20] who stated that there was no noticeable effects in water parameters when carp was fed *Bacillus spp* and probiotics photosynthetic bacteria. This observation on water quality indifference in this experiment could be a result of the liquid faeces which polluted the water in which the fish was raised and this probably may mask the effects on water quality from proper observation.

Haematological parameters observed in this study presents a better concentration of Hb, RBC, WBC, MCV, MCH and MCHC when compared with control diet. This observation indicates that probiotics supplemented diets were more stable health wise than control diets probably due to decrease in cortisol levels in the blood plasma as stated by Rollo *et al* [17] in sea bream (*S.aurata*). The RBC indices like MCHC which denoted the normal corpuscular haemoglobin concentration was reported by Johnson *et al*. [23] and George & Parker [22], to have the mean range of 32-36g/DL and MCV of 80-100μm^{-3}. The result obtained were within range and are significantly ($P \leq 0.05$) higher in the probiotic based treatment. This also support the facts that fish fed probiotics diets were healthier [21].

VII. Conclusion

From the results presented in this research work, we conclude that growth performance and nutrient utilization were significantly better in treatment groups where commercial probiotics (MY-500 (MitoYeast)) and developed probiotics were supplemented than in fish fed the control diet. It was also affirmed that locally developed probiotics are comparably better than commercial probiotics due to the fact that it was isolated from the intending host. Furthermore, water quality parameters observed were within same range in all treatments including control. The haematological parameters observed confirmed that probiotics are capable of improving the health status of its host when included in their diet and this effect improved the immune systems of fish fed probiotics and thereby enhance their physiological status.

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