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Research Paper

Effect of Probiotic (Lactic Acid Bacteria) On the Growth of Fingerlings of *Clarias gariepinus*

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ABSTRACT: *This experiment was carried out to evaluate the effects of the probiotics (Lactobacillus) on the growth performance of African catfish Clarias gariepinus fingerlings. Five experimental diets containing 50% crude protein were used. The control diet was without probiotic supplementation whereas the second, third, fourth and fifth were laced with probiotics at different concentrations of 2%, 4%, 6% and 8% representing diets 2, 3, 4, 5 respectively. Fingerlings of African catfish Clarias gariepinus were divided into 5 treatments with replicates and reared in 100 litre plastic tanks. Twenty (20) fingerlings were distributed per plastic tank and were fed with the different experimental diets at 5% body weight, twice daily for 56 days. The mean weight of fish and water quality parameters were measured weekly. The mean initial weight was not significantly different (p>0.05). The Mean Final Weight of the fish differed significantly (p<0.05) with values ranging from 13.64g (diet 1) to 34.01g (diet 5). There was a significant difference (p<0.05) in the mean weight gain with a range from 12.42g in diet 1 to 32.78g in diet 5. The specific growth rate also differed statistically with the lowest value recorded in Diet 1 (4.99%/day) and highest value obtained in Diet 5 (6.78%/day). The feed conversion ratio differed significantly (p<0.05) across treatments and ranged from 1.13 in diet 5 to 1.39 in diet 1. The protein efficiency ratio also differed significantly (p<0.05), ranging from 1.71 in diet 1 to 2.10 in diet 5. The percentage survival recorded in the experiment was least (90.00%) in diet 4 and highest (100.0%) in diet 5. Probiotics (Lactobacillus and Leuconostoc) in the diet of C. gariepinus has been shown to improve growth performance above non-inclusion. It is therefore recommended that farmers should include the probiotic in diets for their fish at 8% level of supplementation.*

KEYWORDS: *supplementation, diets, catfish, probiotics, lactobacillus*

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I. INTRODUCTION

The intensification of aquaculture requires cultivation at high densities [1], which has caused significant damage to the environment due to discharge of concentrated organic wastes [2, 3], that deplete dissolved oxygen in ponds [4]. Moreover, under intensive production, aquatic species are subjected to stress and this increases the risk and incidence of diseases that results in low productivity.

Crop loss and other losses that are associated with outbreaks of viral, bacterial, and fungal infections within the culture system can be huge especially when it occurs in cage culture systems. Instances of losses reported at the international level include those of China in 1993 which amounted to $750 million and India between 1995 and 1996 where $210 million worth of loss was reported. This notwithstanding, remarkable stock mortality orchestrated by poor environmental conditions on farms [5], unbalanced nutrition [6], generation of toxins [7], and genetic factors [8] also account for poor productivity and losses. Prevention and control of aquatic animal diseases in modern aquaculture relies heavily on the use of chemical additives and veterinary medication, especially antibiotics [9], which generate significant risks to public health by promoting the selection, propagation, and persistence of bacterial-resistant strains [10].

According to Fuller [11], a probiotic is either live microbial assemblage as feed supplement or a cultured product of micro-organisms with beneficial effect on a host through improved balance of intestinal biota. From the foregoing, it is clear that there is need to have two biological entities: the live micro-organism and a host. Probiotics were used initially to either maintain or re-constitute the balance between friendly and pathogenic microorganisms that exist in the gastro-intestinal tract (GIT) or mucus lining on the skin of aquatic

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organisms. It is noteworthy that some bacteria that have been considered for use as probiotics also had antiviral effects [12]. Research towards understanding the mechanism by which these bacteria execute antiviral properties is under study [13].

It is clear that there are direct effects of ecological factors on microbial growth and activity [14] with similar disposition on the GIT of the aquatic animals [15, 16] as well as their feed [17]. Therefore, pathogenic bacteria such as *Aeromonas hydrophila*, [18], *Edwardsiella tarda* [19], *Vibrio parahaemolyticus* [20] and other bacteria species [21] remain at the core of interventions using probiotics. The efficacy of the probiotics also depends on endogenous ecological factors such as pH and redox potential, water activity and nitrite as well as exogenous factors that include temperature and water with specifications that aim to minimize the risk of growth of these organisms [22]. In addition, it is clear that microbial food webs are integrated within the aquaculture system regardless of types of units used: ponds, tanks, cages etc., and these have a direct impact on productivity regardless of intensity of feeding [23]. Therefore, all probiotics prepared with microorganisms have important roles in pond aquaculture, considering productivity, nutrition, health, water quality, and quality of aquaculture effluent. With the foreground in mind, this study was designed to evaluate the growth response of *Clarias gariepinus* fed different dietary levels of probiotics (*Lactobacillus*) with consideration of water quality observed during the trials.

II. MATERIALS AND METHODS

Source of Fingerlings

The fingerlings of *Clarias gariepinus* were obtained from PTERAS Integrated Farm in Makurdi, Benue State, Nigeria and the experiment was conducted at the Fisheries and Aquaculture Department hatchery room North Core of the Joseph Sarwuan Tarka University, Makurdi.

Preparation of Probiotic (*Lactobacillus and Leuconostoc*)

Unpolished rice weighing 250 grams was measured and washed with filtered water of 500ml. washing period was about 5 minutes and after the rice was sieved and the water was collected and stored for 7 days and after which a litre of liquid milk was mixed together with the rice water and stored for another 7 days. The mixture was kept in a dark room during this process. On the 7th day, the solid layer was removed and probiotics was collected. Brown sugar (300g) was added and mixed with the probiotics for preservation. Identification and isolation of the species of lactic acid bacteria present in this formulation was done using methods described by Oyedeji, et al. [24]. *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *L. cellobiosus*, *L. plantarum* and *Leuconostoc lactis* were dominant in the product.

Experimental Feed

Commercial feed (Coppens ®, 2.0mm) was purchased and used as base feed. The treatments were processed using 1.5kg base feed weight (Diet 1) with probiotics added accordingly to make up for the weight (Table 1). Surface application was utilized and the feed was then sun-dried, packaged and stored (refrigerator) for use. The feed was laced with different levels of probiotics at 0%, 2%, 4%, 6% and 8% representing diets 1, 2, 3, 4 and 5 respectively as shown in Table 1.

Table 1: Dosing of probiotics into feed

Diet	Probiotic Level (%)	Feed Weight (kg)	Probiotic Weight (kg)
DT1	0	1.5	0
DT2	2	1.47	0.03
DT3	4	1.44	0.06
DT4	6	1.41	0.09
DT5	8	1.38	0.12

Experimental Set-up and Management

Ten plastic tanks (100 litre capacity) were filled with 50 litres of de-chlorinated water and stocked at 20 fish per tank (completely randomized design having five (5) treatments in triplicates). The fish were fed at 5% body weight twice daily. The mode of feeding was manual by spot feeding into the tanks. The mean initial weight of the fingerlings in each tank was 24.61g ± 0.20. To maintain good quality of water, the water was changed every week. The fish were weighed at the beginning of the experiment and after every week for the period of 8 weeks.

The quantity of feed was adjusted based on the new body weight of the fish in each tank. Mortalities were recorded accordingly.

Water Quality Parameters

Water electrical conductivity, total dissolved solids (TDS) and pH was determined using the Hanna Instruments HI98129 meter while dissolved oxygen was determined using the Hanna Instruments HI9147 portable dissolved oxygen meter. Water temperature was determined using a mercury in glass thermometer.

Data Collection

At the end of the experiment, the biological parameters were calculated using the formulae:.

$$Mean\ Weight\ Gain\ (MWG) = Mean\ Final\ Weight\ (MFW) - Mean\ Initial\ Weight\ (MIW)$$

$$Specific\ Growth\ Rate\ (SGR\ \%/day) = \frac{LnW_2 - LnW_1}{t_2 - t_1} \times 100$$

Where; W_1 = Initial weight of fish at time t1

W_2 = Final weight of fish at time t2

$t_2 - t_1$ = Duration (in days) considered between W_2 and W_1

Ln = Natural logarithm

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Weight\ of\ Dry\ feed\ intake}{Wet\ Weight\ Gain}$$

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{Wet\ Weight\ Gain}{Protein\ fed}$$

Where; $Protein\ Fed = \frac{\%\ Protein\ in\ diet \times Total\ diet\ consumed}{100}$

$$\%\ Survival = \frac{Total\ number\ of\ fish - Mortality}{Total\ number\ of\ fish} \times 100$$

Data Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) at 5% level of significance. Mean separation was done using Fisher's least significant difference (LSD). Data was analysed with the aid of SPSS version 21.

III. RESULTS

The growth performance indices of African catfish *Clarias gariepinus* fingerlings fed different dietary levels of probiotics (Lactobacillus) is shown in Table 2. The mean initial weight (MIW) was statistically the same ($p>0.05$) for all treatments (Table 2). The growth performance indices of African catfish *Clarias gariepinus* fingerlings fed different dietary levels of probiotics (*Lactobacillus*) reveals that Mean Final Weight (MFW) differed significantly ($p<0.05$) across the treatments with the highest value in diet 5 (34.01g) and the least value in diet 1 (13.64g). The Mean Weight Gain (MWG) also differed significantly ($p<0.05$) across treatments ranging from (12.42g) in diet 1 to (32.78g) in diet 5. Specific Growth Rate SGR differed significantly ($p<0.05$) across treatments. The highest value of the SGR was recorded in diet 5 (6.78%/day) and the least value was obtained in diet 1 (4.99%/day). Similarly, there was a significant difference ($p<0.05$) in Feed Conversion Ratio (FCR) across treatments where the highest value of the FCR was recorded in diet 1 (1.39) and the lowest value of FCR were obtained in diet 5 (1.13). The PER also differed significantly ($p<0.05$) across treatments ranging from (1.71) in diet 1 to (2.10) in diet 5. There was a significant difference ($p<0.05$) across treatment in percentage survival ranging from (90.00%) in diets 1 and 4 to (100.0%) in diet 5.

Table 2: Growth Parameters of *Clarias gariepinus* Fingerlings Fed Diets of Probiotics at Different Inclusion Levels

Parameters	DT1	DT2	DT3	DT4	DT5	P-Value
MIW	123.±0.004	1.23±0.007	1.23±0.004	1.23±0.004	1.23±0.004	0.74
MFW	9.94±0.07^{a}	18.34±0.06^{d}	16.88±0.14^{c}	14.678±0.13^{b}	21.13±0.13^{e}	1.40×10^{-8}
MWG	8.72±0.07^{a}	17.11±0.05^{d}	15.64±0.13^{c}	13.44±0.13^{b}	19.90±0.13^{e}	1.18×10^{-8}
SGR	4.99±0.01^{a}	6.44±0.01^{d}	6.24±0.01^{c}	5.91±0.01^{b}	6.78±0.01^{e}	1.03×10^{-9}
FCR	1.39±0.04^{e}	1.19±0.01^{b}	1.24±0.01^{c}	1.27±0.04^{d}	1.13±0.01^{a}	2.67×10^{-4}
PER	1.71±0.05^{a}	2.00±0.01^{d}	1.92±0.01^{c}	1.82±0.05^{b}	2.10±0.01^{e}	1.11×10^{-4}
%Survival	90.00±7.07^{a}	92.5±10.61^{a}	95.00±7.07^{a}	90.00±0.00^{a}	100.0±0.00^{a}	0.56

Means on the same row with different superscripts differ significantly (P<0.05)

Key: MIW = Mean Initial Weight, MFW = Mean Final Weight, MWG = Mean Weight Gain, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, PER = Protein Efficiency Ratio

The weekly growth curve of *Clarias gariepinus* fingerlings fed different diets laced with probiotics (*Lactobacillus*) at different inclusion levels (Figure 1) reveals that diet 5 had the highest growth rate while diet 1 had the lowest growth rate.

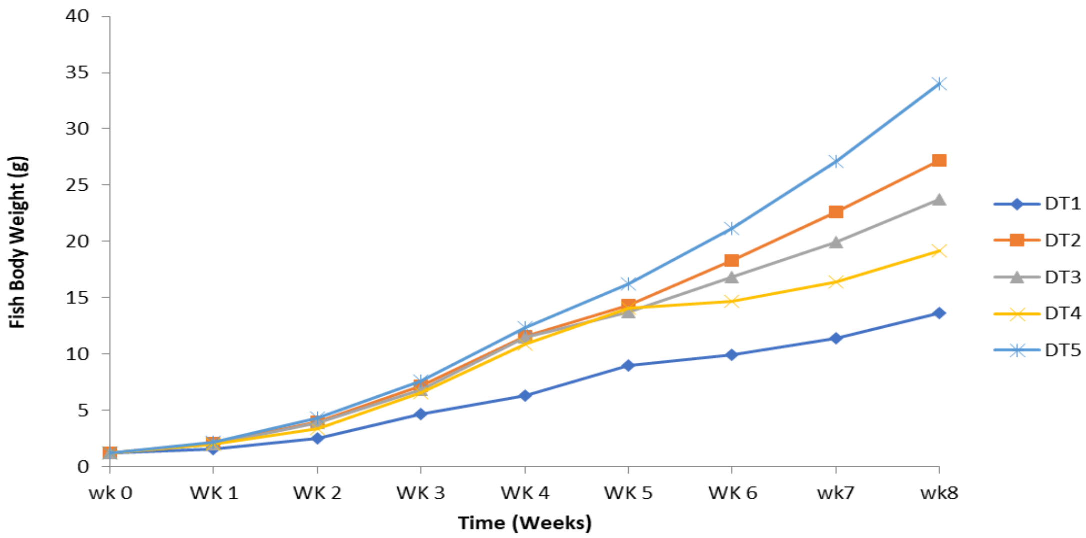

Figure 1: Weekly Growth Curve of *Clarias gariepinus* Fingerlings Fed Diets of Probiotics (*Lactobacillus*) at Different Inclusion Levels.

Table 3 shows the water quality parameters measured during the experiment which revealed that the Electrical Conductivity (EC) of water during the experiment differed significantly (p<0.05) across treatment. The highest EC value was recorded in diet 2 (804.20μs/cm) while the least was obtained in diet 5 (679.20μs/cm). There was a significant difference (p<0.5) in the Total Dissolved Solids across treatments. The total dissolved solids (TDS) had the highest value in diet 2 (401.50mg/l) while the lowest was recorded in diet 5 (339.50mg/l). There was no significant difference (p>0.5) in the Dissolved oxygen level, pH and Temperature across the various treatments

Table 3: Mean Water Quality Parameters Measured During the Experimental Feeding of *Clarias gariepinus* Fed Diets of Probiotics at Different Inclusion Levels

Parameters	DT1	DT2	DT3	DT4	DT5	P-Value
EC (µs/cm)	734.50±43.00^{b}	804.20±55.25^{c}	764.80±31.75^{bc}	773.00±23.50^{bc}	679.20±29.75^{a}	0.50
TDS (mg/l)	369.20±23.25^{b}	401.50±27.00^{c}	381.80±16.25^{c}	386.00±11.50^{c}	339.50±15.00^{a}	0.65
pH	8.72±0.06^{a}	8.72±0.02^{a}	8.72±0.04^{a}	8.71±0.05^{a}	8.73±0.07^{a}	0.96
Temperature (^{0}C)	25.75±0.05^{a}	25.85±0.20^{a}	25.95±0.20^{a}	25.98±0.28^{a}	25.65±0.05^{a}	0.95
DO (mg/l)	4.01±0.05^{a}	4.00±0.13^{a}	4.03±0.13^{a}	4.23±0.08^{a}	4.29±0.01^{a}	0.89

Means with same superscript do not differ significantly (P>0.05)

Key: EC = Electrical conductivity, TDS = Total Dissolved Solids, DO = Dissolved Oxygen

IV. DISCUSSION

The results on the evaluation effects of including different levels of probiotics (*Lactobacillus*) showed significant difference across treatment with best growth performance recorded in fish fed diet with 8% inclusion of probiotic and the least performance was recorded in DT1 which is the control (0% inclusion). The result reveals an increasing trend of growth parameters and feed utilization indices with increasing levels of probiotics and this tallies with the report on the use of probiotics in Nile tilapia (*Oreochromis niloticus*) by Opiyo, et al. [25].

The better weight gain recorded by fish fed diet containing probiotics showed further the evidence of better utilization of the diets by *Clarias gariepinus* compared with the control diet without probiotics [26]. The significant difference in the specific growth rate (SGR) of the fish fed tends to suggest that diet of probiotics is a good ingredient for the growth of *Clarias gariepinus* [27].

Protein Efficiency Ratio (PER) is based on the weight gain of a test subject with respect to its intake of a particular food protein during a test period. It is a measure of growth using the dietary protein as an index and so a high PER value will be obtained if the weight gain is high compared to the dietary protein intake. In the present study, the high mean PER values for the diets containing probiotics tend to suggest that the bulk of probiotics consumed was converted to fish flesh. Diet 5 proved to be the diet of highest quality because it has the highest MWG, PER, SGR values resulting from the presence of probiotics [28]. The lowest FCR obtained in diet 5 is an indication of high quality feed and that it takes less feed to produce high yield [29].

The temporal growth pattern of the fish shows that fish fed diet 5 had the best progression in weight over the duration of the experiment and there was an increasing trend of growth parameters with increasing concentration of probiotics. The present work is in line with the reported work of Opiyo, et al. [25] who reported that the application of *Bacillus subtilis* significantly improved body protein composition of the Nile tilapia. It has been reported earlier that probiotic microorganisms are able to colonize gastrointestinal tract when administered over a long period of time because they have a higher multiplication rate than the rate of expulsion, so as probiotics constantly added to fish cultures, they adhere to the intestinal mucosa of them, developing and exercising their multiple benefits [30].

The mean quality parameters values of water for temperature, dissolved oxygen and pH were within the recommended range for effective fish culture as put forward by Boyd and Tucker [31].

In conclusion, the inclusion of probiotics (*Lactobacillus*) in the diet of *C. gariepinus* has been shown to improve growth performance above non-inclusion. It is therefore recommended that farmers should follow the simple protocol shown in this paper and include the probiotic in diets for their fish at 8% level of supplementation.

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