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Growth Performance and Survival Rate of African Catfish (*Clarias gariepinus*) Juveniles Fed Probiotics (*Lactobacillus acidophilus*) Based Diets

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Abstract: The effect of probiotics (*Lactobacillus acidophilus*) on the growth performance and survival rate of *Clarias gariepinus* juveniles was examined. Four experimental diets (diet 1, 2, 3 and 4) were formulated to contain 0, 1.5×10^8 Cfu/g, 3.0×10^8 Cfu/g and 4.5×10^8 Cfu/g of probiotic (*Lactobacillus acidophilus*) isolated from yoghurt. A total of two hundred and forty (240) African Catfish (*Clarias gariepinus*) juveniles of average body weight of 7.1g \pm 0.54g were used for this study. Each treatment diet was replicated thrice with 20 juveniles per container. The fish were fed twice daily at 5% body weight for 56 days. After the feeding trial, the results showed that the weight gain significantly increased (p < 0.05) with the inclusion probiotic (*Lactobacillus acidophilus*). The highest average weight gain (29.53 \pm 0.73) was observed in juveniles fed diet 3 (3.0 \times 10⁸ Cfu/g). Fish fed diet 3 and diet 2 had protein efficiency ratio of 2.23 g \pm 0.18 and 2.12 g \pm 0.14 respectively which are significantly higher than other test diets. The lowest feed conversion ratio (1.13 \pm 0.04) was also recorded in fish fed diet 3. The study revealed that the survival rate increased with increase in probiotic inclusion, as the highest was recorded in fish fed diet 4 (85%). Based on the results, the study concluded that supplementation of probiotic (*Lactobacillus acidophilus*) in fish feed at an optimum inclusion level improved growth performance and survival rate of *Clarias gariepinus* juveniles.

Keywords: African Catfish; Probiotic; juveniles; growth; survival rate

1. Introduction

Fish is an excellent source of animal protein consumed by the average Nigerian and it accounted for almost 50% of the total protein intake (Ayinla, 2007).^[1] As the production from capture fisheries decline, aquaculture is seen as the best alternative means of increasing domestic production (Oluwatobi et al., 2017).^[2] Aquaculture in Nigeria has gained more popularity over the years, and thereby resulted to wide participation with progressive development in most parts of the country. Despite the progress in Nigeria as one of the leading aquaculture country in Africa, the sector is still bedevilled with constraints which limit it from reaching its full potential (Kaleem and Sabi., 2021).^[3]

Feed constitute more than 60% of production cost in aquaculture (Fedri et al., 2016). Unsustainable feed cost with relatively low conversion rate as well as high risk of disease infestation are among the major factors that limit the growth of aquaculture in Nigeria (FAO, 2007).^[4] Aquaculture requires optimization of nutrition to enhance growth and improve disease resistance of cultured fish for the purpose of food production (Stefanie, 2014).

Researchers have intensified efforts in identifying and developing safe dietary supplements and additives that can enhance growth, health and immune system of farmed fish (Shim et al., 2009).^[5]

Supplementation of fish feed with probiotics has been described as one of the ways to overcome the obstacles faced in intensive fish culture. The probiotics are live bacteria, yeast and fungi, which once supplied in sufficient quantities, give a beneficial effect on the host health (FAO, 2002).^[6] The heavy reliance on the use of antibiotics for combating disease in aquaculture has been observed to have adverse effect and create problems such as accumulation in the tissue and disease resistance in aquaculture (Tukmechi et al., 2007).^[7] Antibiotics alter gut microbiota in terms of viable numbers and diversity (Ige, 2013).^[8] Probiotics have been observed to enhance the growth of beneficial bacterial around the intestinal wall of the fish which ends up improving feed utilization.

Clarias species (Clarias spp) are the most dominant cultured fish species in Nigeria, accounting for about 90% by weight of the fish produced from aquaculture (Igoni-Egweke, 2018).^[9] They are favoured for the growth, feed conversion efficiency and market acceptability (Williams et al., 2012).^[10] Presently, the local demand for the fish keeps increasing with the population; therefore there is a need to increase the production through optimization of nutrition to enhance feed utilization and health. This study is aimed to examine the growth response and survival rate of African Catfish (*Clarias gariepinus*) fingerlings fed probiotics (*Lactobacillus acidophilus*) supplemented diet.



Table 1. Composition of Experimental Diets

Ingredients	Diet I	Diet II	Diet III	Diet IV
Lactobacillus acidophilus	-	1.5 x10 ⁸	3.0 x10 ⁸	4.5x10 ⁸
Fishmeal (kg)	20	20	20	20
Maize (kg)	30	30	30	30
GNC (kg)	23	23	23	23
Soybean (kg)	25	25	25	25
Lysine (kg)	0.1	0.1	0.1	0.1
Methionine (kg)	0.1	0.1	0.1	0.1
Vitamin C (kg)	0.1	0.1	0.1	0.1
Salt (kg)	0.1	0.1	0.1	0.1
Bone meal (kg)	1.0	1.0	1.0	1.0
Premix (kg)	0.6	0.6	0.6	0.6
Total (kg)	100	100	100	100

Table 2. Probiotics Concentration and Viability Test

Feed Treatment	Probiotic	Viability Test	
	Concentration (Cfu/g)	(Cfu/g)	
Diet 2	1.5x10 ⁸	1.1x10 ⁶	
Diet 3	3 3.0 x 10 ⁸	2.1x10 ⁶	
Diet 4	4.5 x 10 ⁸	3.3x10 ⁶	

2. Materials and Methods

2.1. Study Location

The study was carried out at the Fish Biology Laboratory of Oyo State College of Agriculture and Technology, Igboora, Nigeria. A total of two hundred and forty (240) African Catfish (Clarias gariepinus) juveniles of average body weight of 7.1g ± 0.54g were used as the test fish species in this study. The fish were acclimatized for two days before the start of the feeding trials. The fish were distributed evenly in 12 (1x1) plastic container at 20 fish/container, with each of the four (4) treatments having three replicates.

2.2. Experimental Diet

Feed ingredients such as fishmeal, soybean meal, groundnut cake, maize, methionine, lysine, fish premix, DCP were purchased at local feed ingredient store in Igboora. The ingredients were thoroughly mixed appropriately and divided in four (4) experimental diets containing 40% CP, and then pelletized (Table 1). 200 ml of media containing Lactobacillus acidophilus (Probiotics) isolated from yoghurt of about 1.5 x 10^8 Cfu/g, 3.0 x 10^8 Cfu/g and 4.5 x 10^8 Cfu/g were sprayed on the freshly pelletized diet 2, diet 3 and diet 4 respectively. Diet 1 which served as the control diet was not supplemented with the probiotic. The pellets were dried in an oven at 30°C and stored. Bacteria count confirmed the final concentration of live *L. acidophilus* before its use for the feeding trial to be 1.1×10^{6} Cfu/g, 2.1×10^{6} Cfu/g and 3.3×10^{6} Cfu/g for diet 2, diet 3 and diet 4 respectively (Table 2).

2.3. Feeding Trial

Each treatment were labelled diet 1, 2, 3 and 4 respectively and fed accordingly with the corresponding diet. The fish were fed at 5% of their body weight twice in a day (9am and 4pm) for period of 8 weeks. Left-over feed, faeces and mortality in each plastic bowl were siphoned out every day. The water in the plastics was changed with pre-conditioned pipe-borne water every three days. Water parameters such temperature and pH were also measured daily.

2.4. Growth Performance Parameters of the Juveniles

Fish growth and feed utilization efficiency in the experiment was measured weekly and at the end of the experiment. The following parameters were measured:

- Weight gained = final weight- initial weight
- Final Weight Initial weight Specific growth rate (SGR) = -Duration of the Experiment (Days)
- Feed intake Feed conversion ratio (FCR) = Body weight gain
- Survival rate = $\frac{\text{Total number of dead fish}}{\text{Total number of stocked}} \times 100$
- Mean weight gain
- Protein efficiency ratio $\neg \neg = \frac{Prease versus Prease versus Average crude protein feed$
- Feed conversion ratio (FCR) = $\frac{\text{Net protein utilization}}{\text{Protein C}} \times 100$ Protein feed

2.2. Statistical Analysis of Data.

All the data obtained were statistically analysed using analysis of variance (ANOVA) for significant differences in the treatment means, and the mean separation was achieved by using Duncan Multiple Range Test using the SPSS software.

3. Results and Discussions

The use of probiotics in aquaculture has been in various forms Dennis and Uchenna (2016).^[11] For the purpose of this study inclusion in feed was adopted. The research revealed that Lactobacillus acidophilus has the distinct ability to survive a considerable range of heat during drying of pelletized feed as revealed in Table 3.

After 8 weeks of feeding trial, the highest average weight gain was recorded in fish fed diet 3 (29.53g \pm 0.73) with 1.5 x 10⁸ inclusion of L. acidophilus. The Average weight gain increased with increase in probiotics, but reduces at 4.5 x 108 inclusion rate (Diet 4: 23.63g ± 0.61). Analysis of variance revealed that the average weight gain of fish fed diet 3 was significantly higher (P < 0.05) than the fish fed control diet (24.36g \pm 0.71). This is in line with the observation of Ali et al. (2005)^[12] who reported improved growth in tilapia fed feed supplemented with L. acidophilus. The higher average weight gain recorded in the probiotic based diet could be as a result of the intestinal modulation effect of probiotics (Fooks and Gibson, 2002).^[13] Diet 4 with highest probiotic inclusion did not promote weight gain performance as effectively as diet 1 and diet 2. This highlights the importance of evaluating probiotic administration level for maximizing efficacy. However, the feed intake of the fish was not significantly different (P > 0.05). The protein efficiency ratio evaluates the quality of protein. It measures the grams gain per gram of protein consumed. Fish fed diet 3 and diet 2 had protein efficiency ratio of 2.23g \pm 0.18 and 2.12 g \pm 0.14 respectively, which are significantly different (P < 0.05) from the PER of fish fed the control diet (1.90g ± 0.16). The PER results indicated that supplementing diets with recommended level of probiotics inclusion can significantly improve protein utilization in Clarias gariepinus juveniles. This observation agreed with the report of Marzouk et al. (2008)^[14] on the influence of some probiotics on the growth performance of Oreochromis niloticus. Probiotics contributes to optimizing protein use for growth which is the most expensive feed nutrient. The feed



Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Initial Weight	7.07±0.50	7.11±0.51	7.08±0.44	7.13±0.55
Final Weight	31.44±0.97 ^b	35.24±1.21 ^ª	36.61±1.18 ^ª	30.77±0.82 ^b
Average Weight Gain	24.36±0.71 ^b	28.11±0.87 ^{ab}	29.53±0.73 ^a	23.63±0.61 ^b
Feed Intake	32.01±1.56 ^a	33.11±1.72 ^ª	33.37±1.02 ^a	32.71±1.08 ^a
Protein Efficiency Ratio	1.90 ± 0.16^{b}	2.12±0.14 ^a	2.23±0.18 ^a	1.81±0.09 ^c
Feed Conversion Ratio	1.31±0.11 ^a	1.17 ± 0.08^{b}	1.13±0.04 ^b	1.38±0.10 ^a
Specific Growth Rate	0.44 ± 0.02^{b}	0.51±0.01 ^a	0.53±0.01 ^a	0.42±0.02 ^b
Survival Rate	75.00±0.10 ^{ab}	70.00±0.15 ^b	80.00±0.05 ^a	85.00±0.10 ^a

conversion ratio of the control diet (1.31 ± 0.11) was significantly higher than that of diet 3 (1.13 \pm 0.04) and diet 2 (1.17 \pm 0.08). This in turn represents the positive aspect of probiotic supplemented diets. The best FCR recorded in diet $3(1.13 \pm 0.04)$ and diet $2(1.17 \pm 0.08)$ suggested that the inclusion of *L. acidophilus* at required application level improved feed utilization. This result is similar to that obtained by Fahkri et al. (2018) who reported a lower FCR values in Fish fed probiotics treated diets. This means that inclusion of probiotics such as Lactobacillus acidophilus can reduce the amount of feed necessary for growth, and this will result to reduction of production cost which is one of the main goals of a farmer. The specific growth rate of diet 3 (0.53 ± 0.01) and diet 2 (0.51 ± 0.01) were significantly higher (P < 0.05) than that of control diet (0.44 \pm 0.02) and diet 4 (0.42 \pm 0.02). Moreover, the survival rate increased with the increase in Lactobacillus acidophilus inclusion. Diet 2 with the least inclusion of L. acidophilus recorded the lowest survival rate which was significantly lower (P < 0.0.5) than fish fed diet 3 (80.00 \pm 0.05) and diet 4 (85.00 \pm 0.10). Dennis and Uchenna (2016)^[11] studied the influence of probiotics on the survival rate of Clarias spp larvae and found better survival in probiotics treatment (B. subtilis and L. bugaricus) compared to the control diet. This could be as a result of probiotics having the ability to suppress the cell density of the pathogenic bacteria which competes for nutrients and other resources (Queiroz and Boyd, 1998).^[15] Forestier et al. (2001)^[16] also reported that Lactobacillus strain produce antimicrobial peptides which play a crucial role in the innate immunity of the host.

4. Conclusions

From the result of this study, it is logical to conclude that addition of probiotics (*Lactobacillus acidophilus*) in feed at an optimum inclusion level as revealed in the study $(3.0 \times 10^8 \text{ Cfu/g})$ positively increased the growth performance, feed efficiency and survival rate of *Clarias gariepinus* juveniles. Probiotics such as *Lactobacillus acidophilus* is environmental friendly and therefore more beneficial when compared to the use of antibiotics and some other chemical treatments.

Conflicts of Interest

The authors declare no conflict of interest.

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