EVALUATION OF GROWTH AND BODY COMPOSITION OF *Clarias gariepinus* FINGERLINGS FED GRADED LEVELS OF *Enterococcus faecium*

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ABSTRACT

A feeding trial was conducted to evaluate the effect of Enterococcus faecium (Cylatin®), on the growth of Clarias gariepinus. One hundred and fifty C. gariepinus fingerlings (mean weight, 9.13 ± 0.55g) were obtained from Darugo Hatchery, Owerri. They were acclimated for 7days, fed with 2mm Commercial feed (Vital®) in 15L rectangular plastic aquaria. Five diets (CP = 40%) were formulated containing the Probiotic at the concentrations of 0, 0.5, 0.75, 1.00, and 1.25cfu of 100/g feed, designated as T_0, T_1, T_2, T_3, and T_4Enterococcus facecium diets respectively, with T_0 as the control. Ten fish each were randomly assigned into the aquaria and fed with the respective treatment in triplicates at 3% body weight daily for 84days. Mean feed intake for the treatments were 51.94g, 43.35g, 38.14g, 42.85g, and 50.22g for treatments T_0, T_1, T_2, T_3, and T_4 respectively. Results obtained from mean weight gain and specific growth rate for diet T_0 (34.66g and 1.86%) and T_4 (36.34g and 1.92%) were similar (P>0.05). Significant differences occurred amongst the treatments for the FCR, PER, and PPV (P<0.05). The study proved that the commercial Enterococcus faecium can enhance the growth and nutrient composition of Clarias gariepinus fingerlings especially at the higher levels of the Probiotic.

Keywords: Evaluation, Growth, Nutrient, *Enterococcus faecium*, *Clarias gariepinus*

INTRODUCTION

Aquaculture is the world's fastest-growing food industry, with a total output of 59.7 million metric tons (FAO, 2012); but challenges still face this industry; not only the high cost of feeds but also the prevalence of diseases in both the hatchery and culture systems. Antibiotics are used in aquaculture to prevent diseases in fish. These have caused various problems such as the presence of antibiotic residues in animal tissues, the generation of bacterial resistance mechanisms, as well as, imbalance in the gastrointestinal microbiota of aquatic organisms (Nakano, 2007). The European Union (EU) has regulated the use of antibiotics in organisms for human consumption. Consumers today, demand natural products such as probiotics which are friendly bacteria seen as the best option for antibiotics substitution in the aquafeed industry, and the aquatic environment in general (Gonzalez *et al*., 2000). Nwanna *et al*. (2014) stated that Probiotic is not only efficient as a feed supplement in controlling diseases but also enhances the growth and body composition of certain fish (Wang, 2011) amongst which is *Clarias gariepinus*.

Clarias gariepinus (African Catfish) is highly appreciated by most consumers because of the quality of its flesh and some other outstanding features that improve its growth and better economic yield (Pruszynski, 2003). This study was carried out to evaluate the effect of the Probiotic, *Enterococcus faecium* (Cylatin®) on the growth performance and nutrient composition of *Clarias gariepinus*.

MATERIALS AND METHOD

This study was carried out for 84 days in the Fish Farm Compex of the Federal University of Technology, Owerri, Imo State, using plastic aquaria (42 x 30 x 27cm). One hundred and fifty *Clarias gariepinus* fingerlings of mean weight, 9.1±0.55g were obtained from DARUGO Fish Hatchery, Owerri, Imo State, Nigeria, and transported to the Fisheries Research Farm of the Federal University of Technology, Owerri in 15L capacity plastic aquaria filled with borehole water (with a pH value of 6.5 and total hardness of 50mg/L). They were acclimated for 7days before the commencement of the experiment and fed with 2mm pellet-sized commercial feed (Vital®). The fish were randomly assigned to treatments T_0, T_1, T_2, T_3, and T_4 representing 0g, 0.5g, 0.7g, 1.00g, and 1.25g respectively at a minimum Probiotic of 1.0 x 10^9cfu of 100/g feed. The commercial Probiotic, 'Cylatin®' was sourced from Switzerland, while the feedstuffs such as fishmeal, soyabean meal, yellow maize, wheat bran, and additives were purchased from Fidelity Agro Services Limited, Egbu Road, Owerri. Ingredients were analyzed for proximate composition (Table 1) and diets computed using Pearson Square Method. The mixed feedstuff (Table 2) was passed through a commercial pelleting machine to form pellets. They were sun-dried until crispy and stored in well-labeled air-tight black polythene bags based on the treatments to prevent spoilage. Complete randomized design (CRD) was employed in the experiment.

The prepared treatments/diets were also analysed (Table 3) and each treatment/diet was fed to the 10 fish randomly assigned in triplicates.

Statistical Analysis

Data obtained were computed and analyzed using the One-way Analysis of Variance (ANOVA), and differences between means separated using Duncan's Multiple Range Test (Duncan, 1955). Test of significance was done at x = 0.05, using SPSS Version 15, Window 7.

RESULTS

The growth response of *Clarias gariepinus* fingerlings fed diets fortified with different levels of the Probiotic is shown in Table 4. The highest weight gain and specific growth rate were obtained in fish-fed diet T_4, followed by diet T_0, T_3, and T_2 respectively. The weight gain and specific growth rate of fish fed with the control diet, T_0 was not significantly different (P>0.05) from those of diets T_4 and T_3. The best values for Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Productive Protein Value (PPV) all fell within the 1.25g of 100/g feed but this was significantly different (P<0.05) from diets T_1, T_2, and T_3 respectively. The poorest values for FCR, PER, and PPV were observed in diet T_1 (0.05cfu/g). These values were significantly different (P<0.05) from those of diets T_2, T_3, and T_4, except for FCR in treatments T_1 and T_2. Feed intake and Protein intake showed the lowest values for diets T_1 and T_2 and T_3 but were significantly different P<0.05) from those of diets T_4 and T_0.

DISCUSSION

The results suggested that the addition of 1.25cfu of 100/g feed of *Enterococcus faecium* improves the growth performance and feed utilization by *Clarias gariepinus* especially in diet T_4. This was in agreement with the findings of Lara-Flores *et al.* (2003); diets that are supplemented with yeast and microbial mixture improved the growth of *Clarias gariepinus*. Abdelhamid *et al.* (2000) and Nwanna *et al*. (2014) also showed that growth performance and feed utilization were significantly higher in fish-fed Probiotic diets compared with those fed with the control diets, which was in accordance with what was observed in this study. The poor growth of the fed lower levels of the Probiotic (T_1 and T_2) may perhaps be due to the levels which fell below the minimum requirement of the Probiotic for fish growth (1.0 x 10^9cfu). Nwanna *et al*. (2014) reported that Probiotics biodegradeanti-nutritional factors in feeds leading to improvement in their nutritional quality. This may have improved the carcass quality of the fish as observed in this study; that eventually resulted in the deposition of more protein and fat as well as the reduction in the moisture content from the control diet across other treatments of probiotic (Table 5). The crude protein of fish is said to be proportional to the quality of feed fed the fish (Davies, 2005), thiswas true as observed in this study, because the feedwith the highest levels of crude protein corresponded with the carcass protein and fat thus determined as recorded in table 3, which showed crude protein in treatment 4 and control for feed in relation to the table 5 for crude protein and fat of the fish. Probiotics as inherited microflora in the intestinal system, with little or no pathogenic nature, have features that are important for the host's health and well-being (Thirumurugan and Viguesh, 2015). This is true of this study as most of the fish observed were active and healthy throughout the experiment, manifesting improved growth. Mohammed *et al.*, (2007) who also reported that Nile tilapia (*O. niloticus)* fingerlings fed on diets supplemented by probiotic, exhibited greater growth than those of the control. The inability of this particular probiotic to achieve this feat may be attributed to the manufacturer's design that the lower levels of the probiotic 0.5 to 0.75 treatments fall short of the minimum requirement for the growth put at 1.0×10^9cfu. The assertion may equally be to the fact that the number of organisms required or necessary to exert a positive effect on the specific enzyme activity for growth may not have been attained at those lower levels, hence the poor growth of the fish at those levels. However, Efthimiou (1996) found that there was no effect of probiotic on the growth performance of Atlantic salmon fry and dentex respectively. He rather said that the probiotic reduced fish fat content and alleviated the hazardous effects of *Aeromonas hydrophilia* on the fish mortality rate.

CONCLUSION

The results of the 84-day feeding trial of *Clarias gariepinus* with the commercial Probiotic, *Enterococcus faecium* 'Cylatin®' revealed that it could enhance growth and nutrient retention for fish in aquaculture by replacing the antibiotics used for fish. We recommend that the probiotic, *Enterococcus faecium* can be administered to fish as from 1.0x10^9 CFU per 100g feed based on the findings of this study that showed the best growth for fish was at 1.25×10^9 CFU per 100g feed, which was equally better than the control in other growth parameters that were evaluated.

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Table 1: Gross Composition of Experimental Diets Fortified with *Enterococcus faecium Diets*

INGREDIENTS	T_0	T_1	T_2	T_3	T_4
Fish meal	43.35	43.35	43.35	43.35	43.35
Soybean meal	21.67	21.67	21.67	21.67	21.67
Yellow maize	15.73	15.73	15.73	15.73	15.73
Wheat bran	5.00	5.00	5.00	5.00	5.00
Bone meal	4.00	4.00	4.00	4.00	3.50
Vit./Mineral premix	4.00	3.50	3.25	3.00	3.25
Vitamin C	1.00	1.00	1.00	1.00	1.00
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Palm Oil	1.00	1.00	1.00	1.00	1.00
Codliver oil	1.00	1.00	1.00	1.00	1.00
Common Salt	0.25	0.25	0.25	0.25	0.25
Starch	2.00	2.00	2.00	2.00	2.00
Probiotic (cfu100/g feed)	-	0.50	0.75	1.00	1.25
	100	**100**	**100**	**100**	**100**

Table 2: Proximate Composition of Feedstuffs used for Feed Formulation

INGREDIENTS (%)	MOISTURE	CRUDE PROTEIN	ETHER EXTRACT	ASH	FIBRE	NFE
Fish meal	7.20	55.80	9.90	18.60	2.79	5.71
Soybean meal	7.30	40.40	-16.40	5.20	10.50	20.20
Yellow maize	13.90	10.00	5.50	3.20	4.20	63.20
Wheat bran	9.60	15.40	4.70	4.70	13.80	51.80

Table 3: Proximate Composition of Formulated Feed (Experimental Diets)

Parameters (%)	TREATMENTS				
	T_0	T_1	T_2	T_3	T_4
Moisture	5.76	5.15	5.92	9.37	5.62
Crude protein	41.97	39.30	39.67	40.11	40.39
Ether extract	5.51	12.95	11.00	12.64	9.87
Ash	4.66	8.76	10.73	9.72	8.51
Fiber	3.13	2.28	3.00	2.32	3.08
NFE	44.73	36.71	35.60	35.21	38.15
Metabolisable Energy (ME-kcal/kg)	3587	3806	3623	3758	3647

Table 4: Growth Performance and Nutrient Utilization of *Clarias gariepinus* Fed Graded Levels of *Enterococcus faecium* Fortified Diet

Parameters	TREATMENTS				
	T_0	T_1	T_2	T_3	T_4
Initial mean weight (g)	9.20±0.53[a]	9.13 ± 0.55[a]	9.13 ± 0.55[a]	9.13 ± 0.55[a]	9.13 ± 0.55[a]
Final mean weight (g)	43.80±2.02[a]	32.03 ± 0.76[b]	32 ± 2.35[b]	36.10 ± 0.88[ab]	45.47 ± 2.14[a]
Mean weight gain (g)	34.60±3.80[a]	22.90 ± 0.40[b]	23.24±0.01[b]	26.97 ± 3.37[ab]	36.34 ± 0.99[a]
Specific growth rate (g)	1.86±0.05[a]	1.50 ± 0.10[b]	1.51 ± 0.01[b]	1.64± 0.11[ab]	1.92 ± 0.01[a]
Mean feed intake (g)	51.94±0.44[ab]	43.35 ± 2.85[ab]	38.14 ± 1.03[b]	42.85 ± 2.33[ab]	50.22 ± 3.99[a]
Feed Conversion Ratio (FCR)	1.50±0.00[b]	1.89 ± 0.11[a]	1.64 ± 0.016[ab]	1.59 ± 0.05[b]	1.38 ± 0.02[c]
Protein Efficiency Ratio (PER)	1.67±0.05[ab]	1.32 ± 0.01[c]	1.52 ± 0.10[b]	1.57 ± 0.06[b]	1.81 ± 0.20[a]
Productive Protein Value (PPV)	199±0.06[b]	145±0.02[c]	191 ± 0.11[b]	1.97 ± 0.05[b]	237 ± 0.30[a]

Table 5: Proximate Composition of *Clarias gariepinus* after 84-day Feeding Diets with Graded Levels of *Enterococcus faecium*

		TREATMENTS				
Parameters (%)	Initial	T_0	T_1	T_2	T_3	T_4
Moisture content	6.16 ± 0.94	5.25 ± 0.01[a]	5.00 ± 0.06[a]	5.00 ± 0.01[a]	5.20 ± 0.07[a]	4.30 ± 0.03[b]
Crude Protein	56.4 ± 5.51	62.15 ± 0.14[ab]	59.00 ± 0.49[b]	60.65 ± 0.57[b]	61.12 ± 0.03[ab]	65.20 ± 3.80[a]
Ether extract	15.5 ± 1.50	18.00 ± 5.00[a]	15.00 ± 3.00[b]	16.00 ± 3.00[b]	18.00 ± 5.00[a]	19.00 ± 4.00[a]
Ash content	15.5 ± 0.50	12.00 ± 1.00[a]	12.00 ± 0.50[a]	12.00 ± 4.00[a]	12.00 ± 4.00[a]	11.00 ± 2.00[a]
Nitrogen-Free Extract	12.05 ± 0.15	2.60± 0.15[b]	9.75± 0.38[a]	6.35± 0.90[ab]	3.38 ± 0.42[b]	0.75 ± 0.50[c]