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Effect of Probiotics (*Lactobacillus* and *Bifidobacterium*) on Growth Performance and Hematological Profile of *Clarias gariepinus* Juveniles

[1]S.O. Ayoola, [2]E.K. Ajani and [1]O.F. Fashae

[1]Department of Marine Sciences, University of Lagos, Lagos State, Nigeria
[2]Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria

Abstract: This study was carried out to evaluate the use of probiotic (a mixture of Lactobacillus and bifidobacterium species*)* on growth performance and hematological parameters of *Clarias gariepinus* juveniles. Fifteen tanks were used and 10 Clarias gariepinus juveniles (mean weight (14.9±0.83 g) per tank, each in triplicate. Five treatment tanks were fed a diet containing 40% crude protein supplemented with varying inclusion of probiotic comprising about 10^9 colony-forming units per gram of diet (the probiotic diet). Diet T_0 contain 0g probiotic (control diet) while the other groups contain 0.5 g, 1.0 g, 1.5 g and 2.0 g probiotic diet. Results showed that Fish fed with diet T_1 (0.5 g probiotic) had the best growth performance. Weight gain by Fish fed control diet and diet containing 0.5g of probiotic were not significantly different (P>0.05). The final body weight, percentage, weight gain and specific growth rate was positively affected at lowest inclusion of probiotic. Feed conversion ratio of all the treatments were not significantly different (P>0.05). Results showed that there were an increase in the blood parameters of *Clarias gariepinus* fed probiotic diet treatment compare to the control diet but were not significantly different (P>0.05) at the 30 day. There was significant difference (P<0.05) among the treated groups at day 60. The 90 day of study showed that values of all hematological parameter of fish fed different concentrations of probiotic diet did not differ from the values of fish fed the control diet but there was an increase in platelet value of fish fed control diet which ranges from Packed Cell Volume (16.4-26.8%), Hemoglobin content (4.06-9.23gd L^{-1}), Red blood cells (0.88-2.95×10^6µ L^{-1})), White blood cells (171.0-213.µ L^{-1}) and platelets (77.83-322×10^3µ L^{-1}). There were no significant differences (P>0.05) in the Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration, of fish fed different concentrations of probiotic. All blood parameters obtained were between the range of recommended fish blood. It is concluded that using probiotic (especially at 0.5 g) as supplementary feed on *Clarias gariepinus* showed a slight increase in the hematological parameters compared with the control diet but it has no negative impact on the health status of the studied species. However, probiotic (Lactobacillus and bifidobacterium) can be used as a probiotic agent in aquaculture, to enhance fish health, survival and growth performance.

Key words: Bio-Indicator · Probiotics · Growth Performance · Heamatology · *Clarias gariepinus*

INTRODUCTION

Aquaculture practices involved using high-quality feeds with high crude protein content which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and result in good growth performance. Aquaculture industry is faced with the challenges of inadequate supply and high cost of quality fish feeds. Commercial fish feed usually containing fish meal as the major protein source, ranging from 30-50% [1]. Beside the problem of high cost of fish feeds and quality seed, disease outbreak is a major challenge in fish farming in Nigeria. Antibiotics had been frequently used to enhance growth and/or resistance to disease in aquaculture systems. The traditional use of antibiotic as growth promoters in aquaculture has been challenged because of the potential development of antibiotic-resistant bacteria, the presence of antibiotic residues in food production, the destruction of microbial populations in the aquacultural environment and

Corresponding Author: S.O. Ayoola, Department of Marine Sciences, University of Lagos, Lagos State, Nigeria. Tel: +234(80)34650102.

the suppression of the aquatic animal's immune system [2]. This criticized the use of these substances to stimulate growth and treat bacterial diseases in most animal industries. Efforts are still being made to develop alternative or supplementary methods to improve fish health. Various studies showed that farmed fish performance might be elevated by using feed additive such as aromatic plant extracts including spices, digestive enzymes and probiotic. Spice and natural herbs such as marjoram, basil, licorice root, black seed and peppermint have been shown to be beneficial by El-Dakar *et al*. [3]. Functional additive, like probiotics, is a new concept on aquaculture [4] where the additions of microorganisms on diets show a positive effect on growth caused by the best use of carbohydrates, protein and energy [4].

Probiotics were originally incorporate into feed to increase the animal's growth and improve its health by increasing its resistance to disease [5]. The uses of probiotic have been studied most extensively in pigs [6]. Probiotic, which are micro-organisms or their products with health benefit to the host, are used in aquaculture as dietary supplements and as a means of disease control. Studies have been focused on growth promotion of fish by probiotic supplements [7] as well as physiological and immune responses of fish by probiotic supplements [7]. The Food and Agricultural Organization (FAO) have stated that there is potential for probiotic foods to provide health benefits and that specific strain is safe for human use [8].

Clarias gariepinus are characterized by the ability to grow on a wide range of artificial and natural foods, attainment of a large size within short time, high yield potentials, hardness and tolerance to low dissolved oxygen and other aquatic conditions [9].

In Nigeria, catfish is widely cultured [10] because of its high growth rate, ability to withstand stress, disease and ability to spawn easily. *Clarias gariepinus* is one of the most suitable aquaculture species in Nigeria and appreciated in a wide number of Africa consumers. The use of antibiotics in aquaculture has been banned in some country due to rejection of export consignment of marine product. Feed additive requires in Nigeria aquaculture should provide a mechanism that would benefits not only the nutrition growth rate of fish but also its health and welfare in modern day fish farming. Functional additive like probiotic is a new concept in aquaculture [4] where the additives of microorganisms on diets show positive effect on growth caused by carbohydrate, protein and energy [11].

In recent years, increasing researches demonstrated the potential benefits of probiotic in aquaculture ponds that include improvement of water quality, enhancement of nutrient of host species through the production of supplemental digestive enzymes, lower incidence of disease and greater survival, improving growth and immune response.

The beneficial effect of the application of certain beneficial bacteria in human, pig, cattle and poultry nutrient has been documented. However, the use of such probiotic in aquaculture is a relatively new concept.

However a lot of researches have not been done on the use of probiotic as feed supplement. This study is to determine the effect of *Lactobacillus* and *Bifidobacterium* species mixture on growth survival and nutrient utilization of *Clarias gariepinus* juveniles and also to determine a harmless dose of probiotic concentration for *Clarias gariepinus* juveniles. This research study will also investigate the effect of probiotic on the hematological profile of *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

Experiment Design: The experiment was conducted for 90 days at the Eco–toxicology laboratory, Department of Marine Sciences, University of Lagos. A total number of 150 catfish juveniles (*Clarias gariepinus*) were obtained from Sabina fishery enterprise and transported in an open 50 litres plastic container to the laboratory. The obtained fish was apparently healthy and free from any infection and were placed in rectangular plastic tanks with 50.5 x 32.5 x 22.5 cm. After two weeks of acclimatization period, fish with an average body weight of 14.9±0.83g were divided into five groups. Each tank was washed and disinfected before the introduction of fish. Ten fishes were randomly distributed in each tank with triplicate. Each plastic tank was filled with dechlorinated tap water. Cleaning and replacement of water in the aquaria was done every day. Fish were fed two times daily to satiation. Five experimental diets were prepared to evaluate the effect of 0g, 0.5g, 1.0g, 1.5g and 2.0g probiotic on growth performance and hematological parameters of catfish juveniles. All the groups were fed with their respective diet twice daily for 90 days. At the end of every week, the weights of each experimental fish were determined using weighing scale (OHAUS MODEL Cs 5000, CAPACITY 5000×2g). This was done by placing a container on the scale and the balance adjusted to zero, after which

Table 1: Gross compositions of experimental diet (100/g) feed

Ingredients	Control (T_0)	T_1	T_2	T_3	T_4
Fish meal (65%)	23.9	23.9	23.9	23.9	23.9
Soya bean meal (45%)	23.8	23.8	23.8	23.8	23.8
Groundnut cake (48%)	24	24	24	24	24
Indomine	12.8	12.8	12.8	12.8	12.8
Maize	13	13	13	13	13
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Mineral premix	1.5	1.5	1.5	1.5	1.5
Probiotic	-	0.5	1.0	1.5	2.0

T_0 - control T_1 Treatment 1 T_2 Treatment 2 T_3 Treatment 3 T_4 Treatment 4

the fishes in each tank were collected by the use of a net into the container and measurements were taken. The water parameters which includes pH, temperature and dissolved oxygen were also measured and recorded.

Experimental Diet: Commercial feed was obtained and a commercially available probiotic was purchased from Forever Living Product Company at Ikeja, Lagos. The treatment tank (T_0) considered as a control which was fed without probiotic, while groups T_1, T_2, T_3 T_4 was included with probiotic and added at 0.5 g, 1.0 g, 1.5 g, 2.0 g levels respectively. The dry ingredients (consisting of fish meal, soya bean meal, groundnut cake, indomine, maize, vitamins and minerals premix) of the experimental diets was thoroughly mixed and made in a pellet form by addition of boiled water (Table 1). The paste was then extruded through a commercial pelletizing machine. The resulting spaghetti like diet (2.0 mm diameter) was air dried and store in plastic.

Growth Parameters

Weight Gain: Weight gain was determined between the final weight and initial weight of experimental fish. Weight gain = Final weight - Initial weight

Specific Growth Rate: It is the percentage rate of change in the logarithmic body weight and was computed.

$$SGR = \frac{Log_{e\ final\ weight} - Log_{e\ initial\ weight}}{Time\ (days)}$$

Percentage Weight Gain: The% weight gain was determined as the difference between the final weight and initial weight of experiment fish

$$\%\ weight\ gain = \frac{Final\ weight\ -\ Initial\ weight}{Initial\ weight} \times 100$$

Feed Conversion Ratio: This was calculated using the formula

$$FCR = \frac{Feed\ fed}{Fish\ weight\ gain}$$

Protein Efficiency Ratio: It is calculated from the relationship between the increment in the weight of fish (i.e weight gain of fish) and protein consumed.

$$PER = \frac{Mean\ weight\ gain\ (g)}{Protein\ intake}$$

Protein Intake:

$$PI = Feed\ intake \times \%\ of\ protein\ in\ diet$$

Percentage Weight Gain:

$$PWG = \frac{Mean\ weight\ gain\ (g)}{Mean\ initial\ weight} \times \frac{100}{1}$$

Proximate Analysis: The proximate composition for experimental diets and fish carcass were measured according to AOAC [12] method.

Hematology Determination: Blood samples were collected thrice from the experimental fishes at the 30,60 and 90 day of the experiment. The blood samples were collected from the caudal peduncle with the aid of a 2 ml plastic syringe caudal vein using 5ml ethylenediaminetetraacetate (EDTA) as anticoagulant [13] Blood, 2.0 ml, was decanted in heparinzed bottles for determination of blood parameters. A compact Sysmex KX-21 was use to run the samples in whole blood mode and pre-dilute mode. The hematological parameters analyzed include White blood cells (WBCs), Red blood cells (RBCs), Hemoglobin (HGB), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Platelets count (PLT).

Statistical Analysis: Data were statistically analyzed using one-way analysis (ANOVA) of variance. Duncan's Multiple comparisons among means were made when significant F- values were observed ($P < 0.05$), using SPSS 18 statistical program.

RESULTS

Proximate Composition of Experimental Diets: The proximate composition of the experimental diet is presented in the table 2. It includes Fish meal, Maize, Soya bean meal, Groundnut cake, Indomine, Vitamin and Mineral premix and Probiotic mixture.

Table 2: Proximate composition of experimental diet

Proximate analysis of feed	T_0	T_1	T_2	T_3	T_4
Moisture (%)	7.99	7.67	7.50	7.79	7.93
Crude protein (%)	40.51	40.39	40.04	40.11	40.32
Lipid (%)	6.48	6.38	6.84	6.61	6.53
Crude fiber (%)	6.4	5.0	5.9	5.4	5.6
Ash (%)	6.88	7.44	7.33	7.29	7.18
% N. F. E	34.74	35.12	35.39	35.8	34.74

Table 3: Growth performance of *Clarias gariepinus* juveniles fed with diets of different levels of Probiotics

Parameters/Treatment	T_0	T_1	T_2	T_3	T_4
Initial weight (g)	14.3±0.48[a]	14.6±0.56[a]	13.3±0.14[a]	14.9±0.83[a]	14.9±1.42[a]
Final weight (g)	50.4±1.68[b]	52.8±5.39[b]	31.0±1.54[a]	43.3±6.27[ab]	49.7±5.89[b]
Weight gain (g)	36.1±1.53[b]	38.1±4.82[b]	17.7±1.40[a]	28.3±5.56[ab]	34.8±5.57[b]
Specific growth rate (%)	1.40±0.04[b]	1.41±0.07[b]	0.92±0.08[a]	1.16±0.10[ab]	1.32±0.10[b]
Feed conversion ratio	2.46±0.10[a]	2.59±0.36[a]	3.49±0.26[a]	3.07±0.58[a]	2.68±0.37[ab]
Protein efficiency ratio	1.01±0.04[a]	1.00±0.11[a]	0.72±0.05[a]	0.87±0.17[a]	0.97±0.15[a]
Percentage weight gain (%)	252.3±12.7[b]	258.9±23.7[b]	132.5±9.02[a]	187.1±28.9[ab]	231.8±32.1[b]
Protein intake	35.5±0.00	37.9±0.00	24.4±0.00	32.2±0.00	35.6±0.00
Survival (%)	100	100	100	100	100

[1]Pooled standard error; Mean in the same row with the same superscript are not significantly different from each other

Table 4: Chemical composition of the whole carcass of African catfish *Clarias gariepinus* after 90 days of feeding diets supplemented with Probiotic

Treatment	T_0	T_1	T_2	T_3	T_4
Moisture (%)	8.20	8.88	8.43	9.40	9.73
Lipid (%)	12.7	12.5	12.2	12.6	11.8
Ash (%)	4.34	4.78	4.50	4.56	4.49
Crude protein (%)	64.9	65.6	64.3	61.5	61.7

Growth Performance of *Clarias gariepinus* Juveniles Fed with Diets of Different Levels of Probiotics: No mortality was detected for *Clarias gariepinus* after 90 days of feeding diets with and without probiotic supplementation; this may be due to the fulfillment of dietary requirement, all water quality parameters were within the acceptable range for African catfish *Clarias gariepinus*. The water temperature was 27.47±0.55°C, dissolved oxygen (DO) from 6.60±0.62 mg L^{-1} and pH from 7.32±0.36

The growth response of *Clarias gariepinus* fed with different levels of probiotic is shown in Table 3. The results showed that the highest weight gain was achieved with fish fed Diet T_1 followed by diet T_0 T_3 and T_4 (which are the diet containing 0, 1.5 and 2.0 g probiotic respectively) and the lowest weight gain was recorded for diet T_2 (1.0 g active probiotic). The weight gain of fish fed with control diet T_0 was not significantly different (P>0.05) from those fed with Diet T_1, T_3 and T_4 (0.5 g, 1.5g and 2.0 g active probiotic) but significantly different (P>0.05) from fish fed diet T_2 (1.0g probiotic). The highest feed conversion ratio (FCR) was recorded for fish fed with diet T_2 and the lowest was achieved by fish fed with diet T_0 but FCR of all experimental feed was not significantly different (P>0.05). The specific growth rate of fish fed with diet T_1 (0.5 active probiotic) has no significantly different from fish fed with Diet T_0, T_3 and T_4 but was significantly different (P>0.05) from fish fed diet T_2. There were no significant differences (P>0.05) in protein efficiency ratio fed with experimental diet. The percentage weight gain of fish fed with Control diet T_0 was not significantly different (P>0.05) from fish fed with T_1 $T_3$and T_4 but significantly different (P>0.05) from fish fed with diet T_2.

Chemical Composition of the Whole Carcass of African Catfish *Clarias gariepinus* After 90 Days of Feeding Diets Supplemented with Probiotic: Proximate chemical analysis of the whole fish body at the end of the 90 day experimental period is summarized in Table 4. These data indicated that moisture content was higher in fish carcass fed with diet T_4 (1.5 probiotic) and lower in carcass of fish fed with control diet. Highest crude protein was recorded for carcass of fish fed with diet T_1 while the lowest crude protein was obtained in carcass of fish fed 1.5 and 2.0g probiotic. Highest content of lipid was obtained in carcass of fish fed diet T_4, while the lowest lipid content was obtained in carcass of fish fed diet T_0, T_1, T_2 and T_3.

Table 5: Hematological parameter of *Clarias gariepinus* juveniles fed with diets of different level of Probiotics 30 days of the experiment

Treatments/ Blood Parameters	T_0	T_1	T_2	T_3	T_4
WBC ($10^3\mu L^{-1}$)	171.2±12.07^a	210.6±5.13^a	202.5±6.44^a	172.4±35.0^a	154.0±33.3^a
RBC ($10^6\mu L^{-1}$)	1.27±0.32^a	2.30±10.0^{ab}	2.18±0.10^{ab}	1.87±0.24^b	1.50±0.40^b
HGB (gdL^{-1})	5.68±1.41^a	9.15±0.44^a	8.77±0.58^a	7.82±1.62^a	6.92±1.55^a
PCV (%)	14.8±3.55^a	26.8±1.84^{ab}	24.7±1.43^{ab}	22.8±3.15^{ab}	17.3±4.74^b
MCV (fl)	118.7±3.87^a	116.3±3.90^a	112.9±1.79^a	119.7±3.19^a	115.0±1.56^a
MCHC (gdL^{-1})	35.8±1.28^a	35.8±2.57^a	36.6±1.24^a	39.5±2.69^a	38.1±2.18^a
MCH (pg)	47.8±4.61^a	39.7±0.56^a	40.0±1.57^a	53.6±10.8^a	44.2±3.36^a
PLT ($10^3\mu L^{-1}$)	121.3±53.33^a	95.3±34.28^{ab}	112.3±18.58^a	77.83±20.36^a	175.33±44.48^a

[1]Pooled standard error; Mean in the same row with the same superscript are not significantly different from each other

Table 6: Hematological parameters of *Clarias gariepinus* juveniles fed with diets of different level of Probiotics 60 days of the experiment

Treatments/Blood Parameters	T_0	T_1	T_2	T_3	T_4
WBC ($10^3\mu L^{-1}$)	173.8±11.04^a	210.6±5.13^c	202.5±6.43^{bc}	203.9±6.85^{bc}	179.7±13.67^{ab}
RBC ($10^6\mu L^{-1}$)	1.63±0.19^a	2.30±0.23^b	2.18±0.25^b	2.04±.40^{ab}	1.94±0.46^{ab}
HGB (gdL^{-1})	7.08±0.57^a	9.15±0.44^b	8.76±0.58^{ab}	9.23±0.43^b	8.65±0.77^{ab}
PCV (%)	16.4±3.32^a	26.8±1.83^b	24.7±1.43^{ab}	22.8±3.14^{ab}	17.3±4.73^{ab}
MCV (fl)	118.5±2.78^a	116.3±3.89^a	112.9±1.78^a	199.7±3.18^a	115.3±1.30^a
MCHC (gdL^{-1})	38.7±1.97a	34.3±0.94^a	32.8±2.66^a	42.2±7.41^a	46.9±9.55^a
MCH (pg)	46.2±3.10^a	39.7±0.56^a	40.0±1.56^a	52.0±8.99^a	43.2±2.22^a
PLT ($10^3\mu L^{-1}$)	114.5±44.0^a	95.3±34.2^a	112.3±18.5^a	77.8±20.3^a	175.3±44.4^a

[1]Pooled standard error; Mean in the same row with the superscript are not significantly different from each other.

Table 7: Hematological parameters of *Clarias gariepinus* juveniles fed with diets of different level of Probiotics 90 days of the experiment

Treatments/Blood Parameters	T_0	T_1	T_2	T_3	T_4
WBC ($10^3\mu L^{-1}$)	193.1±10.3^{ab}	213.6±20.5^b	171.8±0.8^a	177.7±2.2^{ab}	202.8±5.05^{ab}
RBC ($10^6\mu L^{-1}$)	2.95±0.84^b	2.08±0.39^{ab}	1.63±0.20^{ab}	0.88±0.23^a	1.87±0.22^{ab}
HGB (gdL^{-1})	9.10±0.59^b	8.96±2.16^b	7.20±0.89^{ab}	4.06±0.94^a	7.68±0.98^{ab}
PCV (%)	24.7±1.91^a	23.9±4.43^a	19.8±2.60^a	22.2±0.81^a	23.1±3.12^a
MCV (fl)	101.0±5.89^a	115.0±1.77a	120.1±1.96^a	111.0±2.09^a	117.9±2.01^a
MCHC (gdL^{-1})	35.8±1.28^a	35.8±2.57^a	36.6±1.24^a	39.5±2.69^a	38.1±2.18^a
MCH (pg)	41.5±0.91^a	41.2±2.71^a	43.8±1.20^a	46.58±2.8^a	44.9±2.48^a
PLT ($10^3\mu L^{-1}$)	322.3±80.0^b	194.0±57.8^{ab}	134.6±20.1^a	185.1±6.09^{ab}	240.5±93.11^{ab}

[1]Pooled standard error; Mean in the same row with the same superscript are not significantly different from each other.

MCV - Mean corpuscular volume MCH - Mean corpuscular hemoglobin

MCHC - Mean corpuscular hemoglobin concentration PLT - Platelets count

WBC - White blood cells RBC - Red blood cell count

PCV -Packed cell volume HGB-Hemoglobin

Hematological Parameters of *Clarias gariepinus* Juveniles Fed with Diets of Different Level of Probiotics at 30, 60 and 90 Day of the Experiment: The hematological characteristics of *Clarias gariepinus* fed with different levels of probiotic are shown in Tables 5, 6 and 7.

The hematology sample collected at 30 day showed that the WBCs of all fish fed varying inclusion of probiotic was not significantly different ($p>0.05$), while Fish fed control diet (0% probiotic) has the lowest RBCs count and was not significantly different from fish fed with T_1 and T_2 (0.5, 1.0g probiotic) but significantly different from fish fed with T_3 and T_4 (1.5 and 2.0g probiotic). There was no significantly difference among ($p>0.05$) the HGB, MCV, MCHC, MCH and PLT of fish fed all experimental diet but the PCV of control diet was significant different ($P<0.05$) from fish fed diet T_4.

Blood sample collected 60day during the experiment shows that the white blood cell count of fish fed the control diet was significantly different ($P<0.05$) from fish fed T_4 (2.0g) but was not significantly different ($P>0.05$) from WBC of fish fed with diet T_1,T_2 and T_3 ($P>0.05$). There was no significant difference in WBC of fish fed diet T_1, T_2 and T_3. The red blood cell count (RBC) shows

no significant difference (P>0.05) in the blood parameter of fish fed T_0, T_1, T_2 and T_3 but there was significant different (P<0.05) in the red blood cell of fish fed 2.0g probiotic and control diet. The highest PCV was measured in the blood of fish fed diet T_1 and the lowest was observed in fish fed with control diet but there was no significantly different (P>0.05) from the blood of fish fed diet T_1, T_2, T_3 and T_4. There were no significant differences (P>0.05) in Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Platelets count (PLT) of the blood of all fish fed with varying concentration of probiotic. At the 90 days of the experiment, the highest white blood count was recorded for fish fed with T_1 and was not significantly different from WBCs of fish fed T_0, T_3 and T_4 but significantly different from fish fed diet T_2. The lowest red blood cell was recorded in fish fed with T_3 and the highest was recorded in fish fed T_0 but there was no significant difference in the RBCs of fish fed diet T_0, T_1, T_2 and T_4. The PLT count was high in fish fed control diet and there was no significant difference in blood of fish fed with probiotic diet (0.5, 1.0 and 1.5g). There were no significant differences (P<0.05) in the PCV, MCV, MCH and MCHC of all fish fed varying quantity of probiotic.

DISCUSSION

The values of water parameters recorded during the experiment period are within the acceptable range for aquaculture [14]. However, the optimum growth of African catfish requires temperature, 27-30°C, at least 5 mg/l of dissolved oxygen and 6.5-9.0 pH in the rearing water [15]. During the period of experiment, diet stored before use showed that moulds quickly develop in control diet and there was no mould found in the diet containing probiotic. This showed that probiotic contribute to the destruction of moulds, viruses and parasites in food.

At the end of the experimental period (90), the group of fish fed T_1 (0.5 g) probiotic grew better than the group fed the control diet whereas the final body weight of group of fish fed diet T_0, T_1 and T_4 had significantly (P<0.05) higher final body weight than the group of fish fed diet T_2 and T_3. However the lowest final weight was achieved by group fed diet T_2. Analysis of variance for weight gain followed the same trend as in final body weight gain, suggesting that the addition of 0.5g probiotic enhance the growth performance and feed utilization of *Clarias gariepinus***.** The study result also shows that growth performance, specific growth rate (SGR) and feed conversion ratio (FCR) were significantly (P<0.05) higher in fish maintained on the T_1 of probiotic-supplemented diet compared with those on the control diet. These results are in agreement with the findings of Lara-Flores *et al*. [15] who used three diets formulated for tilapia *Oreochromis niloticus* fries, the first was supplemented at 0.5g with a bacterial mixture containing *Streptococcus faecium* and *Lactobacillus acidophilus*; the second was supplemented at 0.5 g with the yeast *Saccharomyces cerevisae*; and the third, a control diet without supplements with two protein levels being 40% and 27% protein. Lara-Flores *et al*. [16] found that all diets supplemented with yeast and the microbial mixture led to the best growth performance than those fed with control diets. Also Mohamed *et al.*[17] reported that the Nile tilapia (*O. niloticus*) fingerlings fed on diets supplemented by probiotic exhibited greater growth than those fed with the control diet. Mukhopadhyay and Ray [18] also found that greatest attainment in rohu *Labeo rohita* fingerlings fish reared on diet containing sesame seed meal fermented by lactic acid bacteria (*Lactobacillus acidophilus)* than feed contain sesame seed meal. Abdelhamid *et al*., [13] results show that growth performance, specific growth rate (SGR), relative growth rate (RGR), nutrient utilization protein efficiency ratio (PER), feed conversion ratio (FCR) and survival were significantly (P<0.05) higher in fish maintained on the probiotic-supplemented diet compared with those on the control diet, [19] showed that the addition of a gram-positive probiotic bacterium to diets increased survival and growth rate of marine fish larvae (snook, red drum, spotted sea trout and stripped mullet). In contrast, Efthimiou [20] found that there was no effect of probiotics on growth performance observed in Atlantic salmon fry and dentex, respectively. It reduced fish fat content and alleviated the hazard effects of A. hydrophila on mortality rate. African catfish *Clarias gariepinus* survival rate was 100% after 90 days of feeding diets with and without probiotic supplementation; this may be due to the fulfillment of dietary requirement, a healthy fish used in this experiment and the best experimental conditions.

Hematological characteristics have been widely used in clinical diagnosis of disease and pathologies of human and domestic animals. The applications of hematological techniques have proved valuable for fishery biologists in assessing the health of fish and monitoring stress responses. Some of the values were fluctuating due to the condition under which the fishes were kept, the condition based on the fact that the fishes are not in their natural habitat and also because of sizes of the fishes, blood values such as the white blood cell, red blood cell and

hemoglobin value. This was reported by Osuigwe *et al*., [21] that the hematological parameters of fish are affected by a range of factor which includes size, age, physiological status, environmental conditions and dietary regime (e.g. quality and quantity of food dietary ingredients, protein sources, vitamins and probiotics).

The hematology results of the present study showed that WBC, RBC, PCV and PLT were affected by diet treatment. At 30 and 60 day blood of fish fed diet with 0.5g of probiotic have the highest WBCs count. The RBC was higher in fish fed the probiotic diet than control diet at 30 and 60 day but was vice-versa at the 90 day of the experiment [7] found increases in RBCs count, HB value, PCV and WBCs count in fish groups fed with diet supplemented with probiotic ((*B. subtillis* and *Saccharomyces cerevisae*). This shows that probiotic can be considered as an alternative method to antibiotics for feed supplement and health improvement in aquaculture at low inclusion level.

CONCLUSION

The result of the ninety days feeding trial of *Clarias gariepinus* with probiotic (a mixture of *Lactobacillus and Bifidobacterium*) showed that probiotic could be used in fish production. However growth performance and blood parameter (especially white blood cell and red blood cell) could be improve in fish by incorporating probiotic at 0.5 g. It can be deduce from this research that feed incorporated with mixture of *Lactobacillus* and *Bifidobacterium* species can be used as a fish feed in catfish *Clarias gariepinus* culture, to enhance fish health, survival, better feed efficiency and growth performance. However, I recommend more research more work on this probiotic under various conditions and probiotic should be made commercially available for aquaculture use at reasonable price.

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