Effect of Probiotic on Growth Response and Nutrient Utilization of African catfish *Clarias gariepinus*

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ABSTRACT

Lactic acid bacteria (LAB) were isolated from *Clarias gariepinus* and were tested for their probiotic activity. Three hundred juvenile of C. *gariepinus* with mean weight of 50.93 ± 0.16g were randomly selected and distributed into fifteen (15) plastic tanks with three replications for each treatment and fed with probiotic fortified diets of 40% crude protein in the following concentrations: Diet 1- 0cfu/ml (control), Diet 2- 10^3cfu/ml, Diet 3 – 10^5cfu/ml, Diet 4 – 10^7cfu/ml and Diet 5 – 10^9cfu/ml. The experimented fish were fed twice per day at 3% body weight feeding rate for 90 days. Growth, Hematology and plasma biochemical changes in the fish were measured. Challenge test was carried out on the probiotic fed fish with the use of *Pseudomonas aeruginosa* as pathogenic indicator to assess their resistance against the bacterium. The growth of the probiotic fed fish was higher ($p < 0.05$) than the control (without probiotic). Hematology and plasma changes in fish fed with diet 2 – diet 5 were significantly higher ($p < 0.05$) than the control diet. The fish fed with probiotic diet 3 – 10^5cfu/ml had the best growth and highest resistance to the bacterium (*Pseudomonas aeruginosa*).

Keywords: Probiotic, *Clarias gariepinus,* lactic acid bacteria, and hemathology

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INTRODUCTION

The global aquaculture industry currently accounts for over 45% of all seafood consumed. This figure was projected to increase from 75% in the past 20 years (FTU, 2007). In Nigeria, fish coming from aquaculture is about 40% of the total fish production and feed cost account for 80% of the total cost of production (Falaye, 1999; Ayinla, 1999). Apart from this, disease incidence is also one of the major problems facing aquaculture industry as hatchery production loss account for 80% losses during the early developmental stages of fry, juveniles and adult fish. To reduce this losses, the use of synthetic antibiotic drugs has been in used, but the cumulative effects is hazardous to human health and it also reduces micro flora of both the aquatic environment and the intestine of fish itself. It is therefore important to seek for an alternative source which is not harmful and that is the use of probiotic (Gatesoupe, 1999; Verschuere et al, 2000; Fuller, 1989; FAO, 1982). Probiotic are micro-cells additives which when added to fish diets in recommended quality and quantity help to boost growth and build up strong immunity and thereby enhancing their ability to resist diseases and also improve health status of the fish (Irianto and Austin, 2002). Its mode of action is the production of enzymes and inhibitory compounds which aids in digestion of feed and leading to high food conversion, which promote growth and are bacteriostatic and bacteriostatic in nature against pathogenic organisms within the fish system (Thomson *et al.,* 1999) Verschuere *et al.,* 2000 and Carnevali *et al.,* 2006, L_1 and Gathin 2004, 2005; Yanbo and Zirong 2006). Lactic acid bacteria such as *L. acidophilus, L. bulgaricus, L. plantarium, L. fermentum, streptococcus* lactic and *Sachoromyces cerevisiae* (FAO, 2004) have been common strains used as probiotic.

In this experiment, Lactic Acid Bacteria (LAB) was isolated from *C. gariepinus* and was subjected to various biochemical tests. The best among the LAB was varied into different concentrations, at inclusion level of 0.25% and included into 40% crude protein diet fed into juvenile African catfish *C. gariepinus*. Growth response and nutrient utilization was studied for 90 days. At the end of the experiment, the optimum concentration of probiotic that gave the best growth and health status was determined and recommended.

MATERIALS AND METHODS

Sample collection, isolation and identification of probiotics bacteria

Sixty samples of juvenile *C. gariepinus* weighing between 50 – 50.05g were randomly selected from the production pond of the research farm of the Department of Wildlife and Fisheries, University of Ibadan, Oyo state Nigeria.

From the sample, bacteria strains were isolated for probiotic quality. Samples from the gill, intestine and skin were taken and cultured in Man Rogosa and Sharp (MRS) and incubated at 30^0C until when the growth becomes visible. The isolated bacteria were purified by subculture on to maintenance medium consisting of MRS broth with 12% (w/v) glycerol. After the biochemical and morphological analysis, the bacteria were identified according to the methods of Bergey et.al. (1984), Austin and Austin (1993) and AP120E strip system (bio merieux).

The antimicrobial activity

The fish pathogenic indicators used in this experiment (*P. aeruginosa, E. coli, Samonella typi* and *Proteus vulgaris*) were obtained from the Microbiology unit of Department of Botany and Microbiology, University of Ibadan, Nigeria. The pure lactic acid bacteria isolates were examined for its inhibitory effects against the four indicators. The antagonistic activities of these probiotics were examined using a well diffusion assay method according to Schillinger and Lucke, (1989) and Takahiro *et.al.* (1991), the inhibition zone was determined according to Ruiz *et. al*. (1996). A drop of supernatant of the isolates was introduced into wells by sterile dropper. The sterilized plates were earlier seeded with indicator organisms. The plates were then incubated anaerobically at 30°C for 24 h, and then checked for the appearance of inhibition zone which was scored positive if the width of the clear zone was 0.5mm or longer.

Safety tests

The two best lactic acid bacteria isolates that showed best probiotic activity against the pathogenic *P. aeruginosa* were used for safety tests. This was done by using 60 healthy *C. gariepinus* (50 – 50.05g g/fish). The fish were acclimatized for two weeks in an indoor tank and the fish were later divided into 3 equal groups with three replicates per each. Group one serves as control, the fish were injected with 0.3ml of sterile normal saline solution while the other two groups (2 and 3) were intra-peritoneal (IP) injected by 0.3 ml of saline containing 10^7cfu/ml of the best two lactic acid bacteria isolates for 24hr. All groups were kept under observation for two weeks to monitor their mortality and morbidity rates. The fish were subjected to laboratory examination and bacterial re-isolation.

Preparation of probiotic bacteria

The lactic acid bacterium with the best probiotic activity after the antagonistic and safety tests was prepared for inclusion into the diet. The preparation of probiotic bacteria was carried out by inoculating the isolates in MRS broth and

incubated at 30 °C for 48 h, then centrifuged at 3000rpm for 30 min. After centrifugation, the bacteria were washed twice with sterile saline and then added to the bacterial cells till one ml saline contain 10^3, 10^5, $10^7$and 10^9cfu/ml bacterial cells.

Fish feeding experiment

Experimental diets

Five isonitrogenous diets of 40% crude protein were formulated using local ingredients in (Table 1) where the control diets (D1) serve as control without Probiotic cells and diets two to five were fortified with probiotic cells as; D2 (10^3 CFU/ml), D3 (10^5 CFU/ml), D4 (10^7 CFU/ml) and D5 (10^9 CFU/ml) at 0.25% inclusion level. The experimental ingredients were mixed and added to form a paste which was later passed through a pelleting machine using 2mm dice and pelleting was made at a room temperature between 25 -30^{0}C. The pellet was sun – dried on a pallet, and latter packed in a black nylon bags and kept in an air tight container and kept in the freezer at -4^{0}C temperature before use. Diets were prepared at two week interval to ensure that the probiotic cells are not denatured.

Experiment Unit

Three hundred juvenile *C. gariepinus* with an average weight of 50.93± 0.166g used for this experiment were obtained from the research farm of the Department of Fisheries and Wildlife University of Ibadan, Oyo State Nigeria. The fish were acclimatized for two weeks and fed with 2mm COPPENS feed before being randomly distributed into 15 tanks with five treatments having three replications per treatment. A day prior to the commencement of the experiment, the fish were staved to prepare them for the experiment. Later, they were randomly distributed into all the 15 tanks and filled to 100litter of water while the mouth of each tank was covered with netting materials to prevent the fish from jumping out of the tank. The fish were fed at 3% B.W and fed twice per day at 8.30a.m and 4.00 p.m. (Nigerian time) with photo period of 12hr light and12hr dark. The experiment was conducted using semi static renewable method where water inside each tank was drained at two days interval.

The experiment lasted for 90daysduring which water parameters such as temperature, dissolved oxygen, and pH were measured. The water in the tanks were changed at two days interval and filled with fresh water while fish feed was readjusted after two week. Any dead fish in each of the tanks were removed daily. The growth parameters and feed utilization indices were calculated as follows:

Weight gain = W2 − W1 Specific growth rate (SGR) = 100 (ln W2 − ln W1)/T; where W1 and W2 are the initial and final weight, respectively, and T is the number of days in the feeding period Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g);
Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g).

Proximate chemical analysis

The tested diets and whole-fish body, from each treatment, were analyzed according to the standard methods of AOAC (1990) for moisture, protein, fat and ash.

Hematological assessment

Fish were tranquilized with 150mg/l of Tricane Methane Sulphonate (Wagner et. al. (1997) for blood collection. Blood samples of the 5 fish were collected before the commencement of feeding trial (day 0) and at the end of the experiment, blood samples of 5 fish of each tank were collected. The blood samples were collected from the caudal vein by sterile syringe using EDTA-disodium as an anticoagulant. The blood samples were used for determining the erythrocyte-count Dacie and Lewis (1991) and hemoglobin-content Stoskopf (1993). The haematocrit-value (HCT) was calculated according to the formulae mentioned by Stoskopf (1993) and Thrall (2004). Plasma was obtained by blood centrifugation at 3000 rpm for 15 min. The plasma was stored in deep freezer for further biochemical analyses. The plasma glucose was determined according to Trinder (1969). The total protein-content was determined by using biuret method as described by Weichselbaum (1946). Plasma Albumin was estimated calorimetrically according to the method of Dumas and Biggs (1972), while plasma globulin was calculated by mathematical subtraction of albumin value from total protein. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman and Frankel (1957).

Challenge test

Challenge test was conducted according to the method of Austin et. al,. (1995). After the feeding trial, 20 fish of each treatments were divided into two equal groups, the first group was IP injected with pathogenic *P. aeruginosa* (0.3 ml of10^7 cells ml−1) according to Schaperclaus et. al. (1992). The second group was IP injected by0.3 ml of sterile saline as control. All groups were kept under observation for 14 day to record any abnormal clinical signs and the daily mortality rate.

Water analysis

The water temperature, DO and P^H were recorded on daily basis. The water temperature was measured by the use of thermometer, (YST - digital), PH was measured using P^H meter using (Teledo – 320 models U.K). DO was measured with combined digital, YSL model 57, VWR company, New Jersey).

Statistical analysis

All data were analyzed by one –way analysis of variance (ANOVA) using SPSS 15.0 version. Duncan multiple range test (Duncan, 1955) was used to solve differences among treatment means at 5% significant level.

RESULTS AND DISCUSSION

Bacteriological characterization

The physical and biochemical character of the isolates examined at different sites are presented in Table 2. The bacteriological characterization indicated that lactic acid bacteria were identified from all the three sites (the gill, skin and intestine) and are grouped into 5 different strains which are; *Lactobacillus fermentum, L. brevis, L. acidophilus, L. plantarium* and *L. xylosus* respectively. Among the five LAB, *L. fermentum* had the highest percentage (60%), and followed by *L. brevis* (17%), *L. acidophilus* (13%), *L. plantarium* (12%) and *L. xylosus* (10%) respectively - see figure 1.

Antagonistic activity

The antagonistic result is presented in Table 3.The result shows that *L. fermentum* had the widest zone of inhibition (8.99 ± 0.23mm) against *P. aeruginosa*, followed by *L. brevis* against P. vulgaris (8.44 ± 0.73mm), *L. acidophilus* against *E.coli* (8.25 ± 0.46mm) while the least was between *L. acidophilus* and *Salmonella typhi* (2.98 ± 0.07mm). From these result, two best bacteria (*L. fermentum* and *L. brevis)* were selected for further examination in the safety test having the highest zone of inhibition.

Safety test

The safety test result is presented in Table 4. The result showed *L. fermentum* had the best result and was the safest as the survival rate was 100% as against *L. brevis* which was 80% being lower when juvenile *C. gariepinus* selected were interperitoneally (i/p) injected with the selected probiotic (*L. fermentum and L. brevis*). The result in the second examination gave further evidence to select *L. fermentum* as the probiotic to be included in the fish diets for further nutritional study in the experiment.

Water quality parameters during the experiment

Throughout the 90 days feeding experiment, the temperature, dissolved oxygen and pH were not significantly different ($p > 0.05$) both the control and in all the probiotic fed tanks. The temperature ranges between 27.0 – 27.2ºC, dissolved oxygen was between 5.40 – 5.43mg/l while the pH ranges between 6.62 – 6.4

Growth response and nutrient utilization of *C. gariepinus*

The result of growth response and nutrient utilization is presented in Table 5. The mean weight gains (MWG) of all the probiotic fed fish are significantly higher ($p < 0.05$) than the control diet, but among the fish fed with probiotic diets, D3 (48.90 ± 0.02g) had the highest MWG. The feed consumed and the specific growth rate (SGR) followed the same pattern as all the probiotic fed fish during the experiment were significantly higher ($p < 0.05$) than fish in the control diets. The highest feed consumed was recorded in diet 3 (D3) – 230.61 ± 0.2g, while the SGR was equally highest in D3 (0.32 ± 0.02). The protein efficiency ratio (PER) and feed conversion ratio (FCR) of D3 (O.61 ± 0.05; 4.1 ± 0.002) had the best result as their performance was better than all other probiotic fed fish, while the control had the least performance. In all, all the probiotic fed fish during the experiment were significantly higher than fish without probiotic cell in the control tanks.

Influence of probiotic on the proximate composition of *C. gariepinus*.

The result of the proximate composition is presented in 6. The result shows that all the probiotic fed fish had significant increase ($p < 0.05$) in the crude protein, crude fat, than the fish under the control diets as lower values were recorded during the 90 days experiment. The highest value of both crude protein and fat were recorded in fish fed with diet 3 (D3), having 52.3 and 4.87% respectively. The ash content and NFE showed a little deviation from protein and fat content. The ash content of the control diet had the highest value (4.48%) while the probiotic fed fish all had lower values, with the lowest recorded in diet 3 (4.33%). The NFE of both the fish under the control tank the probiotic fed fish were not significantly different ($p < 0.05$) as all had almost value ranging between 51.60 – 51.91%

Physiological parameters.

The result of physiological parameters is presented in table 7.The PCV, Hb and RBC of the probiotic fed fish were significantly ($p < 0.05$) higher than the control as the highest value was recorded in fish fed with D3 - 31.6%,9.7gm/100 and 5.2x10/ml respectively. The WBC of all the probiotic fed fish was higher than the fish fed without probiotic cells in the control diet.

But D3 had the lowest value having19.8x10/ml.

All the ALT, AST, glucose and total protein of all the probiotic fed fish were significantly lower ($p < 0.05$) than all the control diets not fed with probiotic diet.

Challenge test

The result of the challenge test is presented in table 8. The result indicated that all probiotic fed *C.gariepinus* in D2, D3, D4 and D5 all had higher survival rate than the control diet. The fish fed with control diet was 20%, but D4 and D5 had equal percentage of 85%, then followed by D2 (90%), while the highest was recorded in D3 having 95%.

All samples taken from the gill, intestine and skin were subjected to biochemical test according to Austin and Austin (1993), Bergy *et. a*l (1984) the results indicated them as lactic acid bacteria of the species of *L. fermentum, L. brevis, L. acidophilus* and *L. xylosus.* Equally, all the isolates showed an inhibitory characteristic against *Pseudomonas auroginosa* which was used as pathogenic indicator. This result was in accordance with the work done by Hagi and Hoshino (2009), Balcázar et. al. (2008) when both isolated LAB from carp and rainbow trout and all had an inhibitory effect against *Aeromonas hydrophylia* in the *invitro* experiment carried out which made them to be used as probiotic, as they prevented infectious diseases.

The result of the feeding trial experimentall shows that all probiotic fed *C. gariepinus* with *L. fermentum* had higher growth rate than the control diets. The higher growth could be attributed to the addition of probiotic cells which was included at different concentration in all the diets. The observation made in this trial was in agreements with Bucio et. al. (2004), Hagi and Hosino (2009) and Abdel-Hamid and Mohammed (2008) when all probiotic fed fishes had higher growth rates. Similarly, this result is in agreement with Mehrim (2001), Drab, et al (2002), Khatab et. al., (2004), Zong-fu song (2000) and Gatesoupe (2002) as they reported that salmon juvenile and fingerling *O. niloticus* fed on probiotic diets exhibited higher growth rate than the control, without probiotic in their conducted feeding trial experiment. Similar result was also obtained with other probiotic strains included in fish diets of Yellow fail (*Seriola lalandei*) Gatedoupe (1989 and 1991), turbort (*Pseta maxima*) Gatesoupe et al (1989), sea bass Decamp and Moriarty (2006), common carp (*Cyprinuscarpio*), Yanbo and Zrong (2006). Mesalhy Aly et al. (2008) and Wang et al. (2008) reported that the inclusion of probiotic in diets may significantly help to stimulate appetite and improving nutrition through production of vitamins, detoxifying toxic compounds in the diets and helps in breaking down of indigestible compounds.

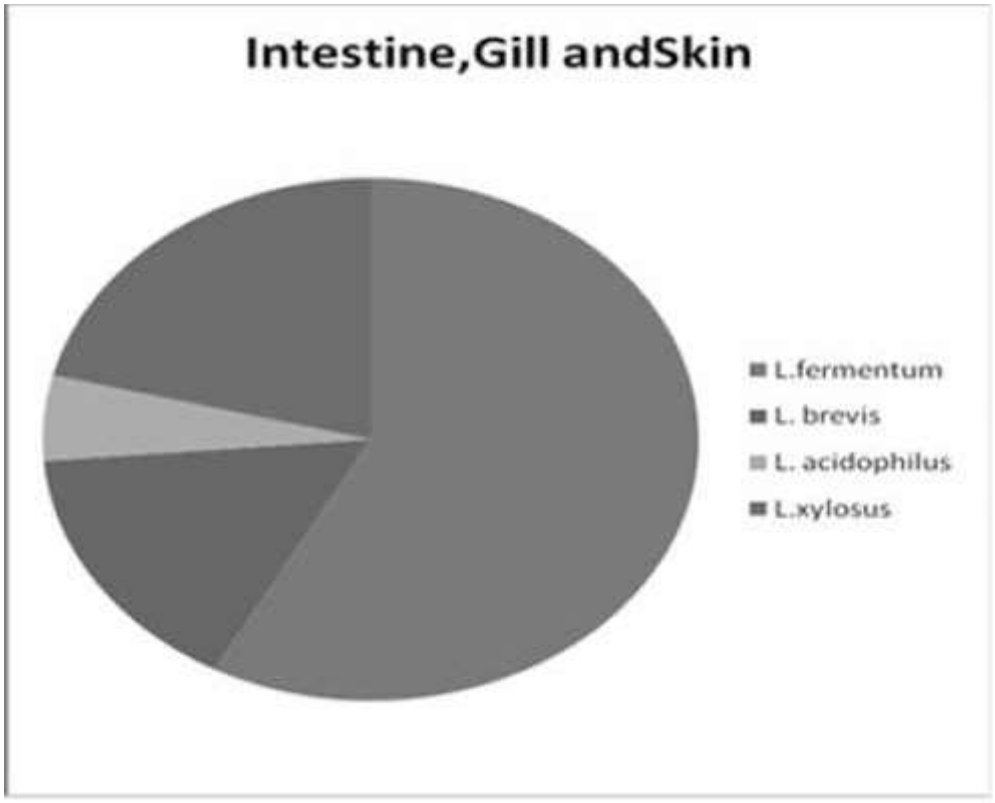

Figure 1: Percentage distribution of identified LAB isolated from *C. gariepinus*

Table1: Influence of probiotic on the mineral composition of *C. gariepinus.*

Ingredients	Diet1 Control	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	20	20	20	20	20
Soya bean meal (SBM)	60	60	60	60	60
Yellow maize	10	10	10	10	10
Vegetable oil	6	6	6	6	6
Vitamin premix	3	3	3	3	3
Starch	1	1	1	1	1
TOTAL	100	100	100	100	100
Probiotic concentration CFU/ml – 0.25% of total diet	0.25	0.25	0.25	0.25	0.25
Chemical composition (%)					
Protein	40.1	40.1	40.1	39.9	40.1
Fat	8	8	8	8	8
Ash	9.5	9.5	9.5	9.5	9.5
Fibre	13.7	13.7	13.7	13.7	13.7
Moisture	12.9	12.9	12.9	12.9	12.9
NFE					
Gross energy (Kcal/g)	497.0	497.0	497.0	497.0	497.0

Fish premix will be purchased from Adom Nig. Ltd

*Vitamin and Minerals: Vitamin A –10,000,000 I.U.; D3- 2,000,000 I.U.; E – 23,000mg; K3 – 2,000 mg; B1 – 3,000 mg; B2- 6,000 mg; Nacin – 50,000 mg; Calcium Pathonate – 10,000 mg; B6 – 5,000 mg; B12- 25.0 mg; Folic acid 1,000 mg; Biotin- 50.0 mg; Choline chloride – 400,000 mg; Manganese – 120,000 mg; Iron- 100,000 mg; Copper– 8,500 mg; Iodine – 1,500 mg; Cobalt-300 mg; Selenium-120 mg; Antioxidant 120,000 mg.

Table 2: Biochemical characterization of Lactic acid bacteria isolated from *C. gariepinus*

Criteria	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Gram reaction	+	+	+	+
Cellular morphology	Rod	Rod	Rod	Rod
Catalase	–	–	–	–
Oxidase test	–	–	–	–
Indole test	–	–	–	–
Starch hydrolysis	–	–	–	–
Motility test	–	–	–	–
MR –Methyl red	+	+	+	+
H_2S production	–	–	+	–
Glucose	+	+	+	+
Lactose	–	–	+	+
Sucrose	+	+	+	+
Salicin	–	+	+	–
Fructose	+	–	+	+
Xylose	+	+	–	+
Raffinose	+	+	+	+
Melibiose	–	+	+	–
Maltose	+	+	+	+
Mannitose	+	+	–	+
Ramanose	–	–	+	–
Galactose	+	+	+	+
Sonbose	–	+	–	–
Trehalose	–	–	+	+
Ribose	–	–	+	+
NH3 from arginine	+	–	+	–
Growth @4%NaOH	–	–	–	–
Growth @ $4^{O}C$	+	–	–	+
Growth @ $15^{O}C$	+	–	–	+
Growth @ $45^{o}C$	+	–	–	+
Homo/ Hetero fermentation	HE	HE	HE	HM
Probable Isolates	*L. fermentum*	*L. brevis*	*L. acidophilus*	*L. xylose*

Table 3: Influence of *L. fermentum* and *L. brevis* on the safety of *C. gariepinus*

Isolate	Control	*L. fermentum*	*L. brevis*
Route of injection	i/p	i/p	i/p
Dosage	0.3ml saline	0.3ml (10^3cell/ml)	0.3ml (10^7cell/ml)
No of fish stocked	20	20	20
Ave. wt. (g)	50.03 ± 0.2g	50.04 ± 0.1g	50.02 ± 0.01g
No of mortality	_	_	_
Healthy	10	20	20
% of survival	50	100	90

Table 4: Growth and nutrient utilization of *C. gariepinus* fed with Probiotic diets for 90 days

Growth parameters	**Treatments**				
Parameters	**D1(Control**	**D2(10^3Cfu/ml**	**D3(10^5Cfu/ml**	**D4(10^7Cfu/ml**	**D5(10^9Cfu/ml**
Initial Mean Wt (g)	50.94±0.16^a	50.66± 0.08^a	50.02± 0.25^a	51.60± 0.20^a	50.44± 0.33^a
Final mean wt (g)	88.08±0.2^d	96.97 ± 0.33^a	99.92± 0.36^a	94.36± 0.61^b	93.29± 0.32^c
Mean Weight gain (g)	37.14±0.31^c	46.31±.21^a	48.90±.02^a	42.76±.4^b	41.85±.03^b
Weight gain (%)	72.91±.4^a	91.41±.01^a	97.76±.01^a	84.51±.05^d	82.96±.04^b
Feed Consumed (g)	183.39±.05^d	196.19±.03^b	230.6±.02^a	196.43±.03^b	194±.03^b
Specific growth rate (SGR)	0.28±.03^c	0.31±.04^a	0.32±.2^a	0.30±.05^a	0.29±.02^b
Protein Efficiency Ratio (PER)	0.51±.03^c	0.59±.04^a	0.61±.05^a	0.54±.03^b	0.55±.05^b
Survival Rate (%)	90	95.02	97	94.5	94.20
Feed Conversion Ratio (FCR)	4.94±.02^c	4.24±.04^a	4.10±.02^a	4.59±.01^b	4.64±.04^b
Nitrogen Metabolism (NM)	3434±.01^c	3649±.04^a	3729±.02^a	3605.9±.04^a	3575.6±.01^b

Mean values with same superscript in row are not significantly different p>0.05

Table 5: The influence of probiotic diets on the proximate composition of *C. gariepinus*

Diets	Crude protein (%)	Crude fat (%)	Moisture (%)	Ash (%)	NFE (%)
D1(Cont.)	50.88±.01^{b}	4.33±.01^{c}	13.05±.01^{a}	4.47±.01^{a}	51.67±.01^{a}
D2	51.23±.01^{a}	4.52±.21^{b}	12.54±.01^{b}	4.47±.01^{a}	51.8±.02^{a}
D3	52.35±.01^{a}	4.87±.01^{a}	12.11±.01^{b}	4.33±.01^{b}	51.95±.03^{a}
D4	51.96±.01^{a}	4.52±.01^{b}	12.12±.01^{b}	4.38±.01^{b}	51.68±.01^{b}
D5	51.49±.01^{a}	4.8±.01^{a}	12.18±.01^{b}	4.37±.14^{b}	51.68±.01^{c}

Mean values with same superscript in row are not significantly different p>0.05

Table 6: Influence of probiotic on the hematology of C. gariepinus before and after being with Probiotic diets for 90 days

Diets	PVC (%)	HB (g/100)	RBC (X10/ml)	WBC (x10/ml)	MCV (fl)	MCH (pg)	MCHC (gm/100)	PLT (X10/ul)
D1(Cont)	21.8±.03^{c}	5.5±.02^{c}	1.91±.02^{c}	11.6±.01^{c}	100±.07^{b}	32.2±.2^{a}	29.6±.03^{b}	232±.1^{a}
D2	28.5±.14^{b}	6.15±.23^{b}	5.1±.03^{a}	42.65±.10^{a}	109±.10^{c}	36.4±.1^{a}	33.5±.04^{a}	133±.0^{5}
D3	31.6±.03^{a}	9.7±.30^{a}	5.2±.02^{a}	19.8±.02^{a}	116.8±.1^{b}	39±.04^{c}	30.7±.02^{b}	268±.02^{a}
D4	21.85±.31^{c}	6.15±.03^{b}	3.85±.12^{c}	30.65±.03^{a}	112.3±.1^{a}	36.3±.8^{a}	31.1±.03^{b}	166±.03^{d}
D5	27.55±.23^{b}	8.2±.03^{a}	3.38±.03^{c}	30.25±.04^{a}	111.5±.1^{d}	36.1±.1^{b}	29.6±.02^{c}	155±.12^{c}

Mean values with same superscript in row are not significantly different p>0.05

Table 6: The influence of probiotic on the plasma biochemistry of *C. gariepinus.*

Diets	Creat. (mg/dl)	AST (u/l)	ALT (Iu/dl)	GLU (mg/dl)	Tt. Protein	Albumin (g/dl)	Globul (g/dl)
D1(Cont.)	1.13±.21^{b}	108±.50^{a}	48.5±.3^{a}	89±.03^{a}	4.05±.1^{a}	1.55±.1^{a}	2.5±.05^{a}
D2	1.15±.05^{a}	81±.03^{c}	40.5±0^{a}	42. ±.2^{c}	3.7±.02^{b}	1.1±.12^{c}	2.6±.02^{a}
D3	1.27±.9^{a}	87±.03^{b}	26±.43^{c}	52.7±.04^{b}	3.8±.05^{a}	1. 65±.1^{b}	2.2±.45^{a}
D4	1.25±.12^{a}	98±.05^{a}	37±.32^{b}	79±.02^{a}	3.5±.02^{c}	1.0±.02^{c}	2.5±.04^{a}
D5	1.22±.02^{a}	103±.03^{a}	43.5±.4^{a}	77±.03^{a}	4.4±.02^{a}	2±.03^{a}	2.5±.02^{a}
Diets	Creat. (mg/dl)	AST (u/l)	ALT (Iu/dl)	GLU (mg/dl)	Tt. Protein	Albumin (g/dl)	Globul (g/dl)
D1(Cont.)	1.13±.21^{b}	108±.50^{a}	48.5±.3^{a}	89±.03^{a}	4.05±.1^{a}	1.55±.1^{a}	2.5±.05^{a}
D2	1.15±.05^{a}	81±.03^{c}	40.5±0^{a}	42. ±.2^{c}	3.7±.02^{b}	1.1±.12^{c}	2.6±.02^{a}
D3	1.27±.9^{a}	87±.03^{b}	26±.43^{c}	52.7±.04^{b}	3.8±.05^{a}	1. 65±.1^{b}	2.2±.45^{a}
D4	1.25±.12^{a}	98±.05^{a}	37±.32^{b}	79±.02^{a}	3.5±.02^{c}	1.0±.02^{c}	2.5±.04^{a}
D5	1.22±.02^{a}	103±.03^{a}	43.5±.4^{a}	77±.03^{a}	4.4±.02^{a}	2±.03^{a}	2.5±.02^{a}

Mean values with same superscript in row are not significantly different p>0.05

Table 8: Challenge test on C. gariepinus probiotic supplemented diets.

Items	D1 (Control)	D2	D3	D4	D5
No of fish injected	20	20	20	20	20
Route of injection	i/p	i/p	i/p	i/p	i/p
Dosage of bacteria	0.3ml of 10^{3}cfu/ml	0.3ml of 10^{3}cfu/ml	0.3ml of 10^{3}cfu/ml	0.3ml of 10^{3}cfu/ml	0.3ml of 10^{3}cfu/ml
Survival rate	4	18	19	17	17
Mortality (%)	80	10	5	15	15
Survival (%)	20	90	95	85	85

*i/p - interperitoneal

The proximate composition of *C. gariepinus,L. fermentum* fortified diets (D2 – D5) fat and protein were significantly higher than the control diet, except the ash content. The increase in proximate composition could be due to higher feed intake which was stimulated by the action of probiotic cells which increased the nutritive value and increased fat deposition and higher protein in all the probiotic supplemented diets fish during this experiment as observed by Irianto and Austin, (2002).

The blood parameters, such as the heamatology and plasma biochemistry are good indicators to determine the health status of any animal. In this experiment there was an increase in the parameters such as the PCV, Hb, and RBC which is an improvement in the fish health under study. This improvement could have been due to the action of *L. fermentum* which increased and improved immunity level of *C. gariepinus* under study.This result confirmed thereport of Abdel et al (2005), Khatab (2004) Majzowk et al (2008), Srikan Jana et al (2008) where various fishes fed with probiotic diets had higher survival rate. The higher survival rate could be attributed to the influence of probiotic cells in the fish diets which increased the immunity level of both fish, hence high survival percentage when compared with fish not fed with Probiotic diets.

The blood plasma of the probiotic supplemented *C.gariepinus* did not increased significantly when compared with control fed fish without probiotic cells. This is an indication that the probiotic fed *C. gariepinus* were not stressed as a result of the influence of probiotic cells fed to all the fishes during the experiment. This result is also an indication that there may be no damage to the kidney and liver of the fish. All the plasma blood in this result agreed with the work of Delanbe et al (1989), Soliman (2000) and Khattab (2007).

The challenge test result is a clear indicator that the probiotic fed *C. gariepinus* had higher survival rate during the experiment when challenged with *Pseudomonas aeruginosa* when compared with the fish under control where highest mortality was recorded. The inclusion of *L. fermentum* in the diets had positive influence on the survival rate of *C. gariepinus* when compared with the fish under control diet (without probiotic cells). This agreed with observation of Abd El-Rhmanet et al, (2009) and Taokaet. al.(2006) that the probiotics-treatment enhanced the nonspecific immune parameters of tilapia such as the lysozyme activity, migration of neutrophils and plasma bactericidal activity, resulting in the improvement of fish resistance against *Edwardsiella tarda* infection. By recommendation from the experiment carried out, the inclusion level of 10^5cfu/ml is an optimum concentration of *L. fermentum* as probiotic for highest survival rate for *C. gariepinus.*

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