Agrosearch, 19(1): 13-27 (2019) https://dx.doi.org/10.4314/agrosh.v19i1.2

GROWTH, NUTRIENT UTILIZATION, HAEMATOLOGY AND BIOCHEMICAL PARAMETERS OF AFRICAN CATFISH (*Clarias gariepinus*, BURCHELL, 1822) FED WITH VARYING LEVELS OF BACILLUS SUBTILIS

*[1]Lawal M.O., [1]Aderolu A.Z., [2]Adewumi G.A. and [1]Mudiaga A.

[1]Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

[2]Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

*Corresponding author: lawdeen2003@gmail.com; (+2348023825115)

ABSTRACT

This study examined the growth response, nutrient utilization, biochemical and haematological properties of *Clarias gariepinus* juveniles, fed with graded levels of *Bacillus subtilis*. Five diets were formulated (35% crude protein; 3127 kCal/kg energy), comprising 0 (T_1), 20 mg/100g oxytetracycline (T_2), 10^5 (T_3), 10^7 (T_4) and 10^9 (T_5) *B. subtilis* CFU/ml. African catfish, *C. gariepinus* (n=150; mean weight =94.33±0.67g) were allotted to 15 rectangular tanks and fed experimental diets apparently to satiation for 8 weeks. Growth performance, nutrient utilization, haematological and biochemical parameters were examined using standard methods. The results showed that fish fed with Diet T_5 recorded significantly high values for mean weight gain (MWG) (116.67±5.70g), specific growth rate (SGR) (1.58±0.07%) and percentage weight gain (PWG) (133.62±7.47%), while Diet T_1 had least values for MWG (89.00±0.58g), SGR (1.36±0.01%) and PWG (94.35±0.91g). Feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly different (p<0.05) across the test diets, with Diet T_5 having the best values for FCR (1.17±0.04) and PER (3.27±0.18). No significant differences (p>0.05) were observed in the haematological, AST, ALP and GSH indices between the fish fed graded levels of probiotic and control diets. The excellent growth performance recorded at the highest inclusion level (T_5) of *B. subtilis* showed that the probiotic could be favourably incorporated into the diet of *C. gariepinus* juveniles.

Keywords: Growth, Nutrient Utilization, Blood Parameters, *Clarias gariepinus*, *Bacillus subtilis*

INTRODUCTION

The farming of catfish is important in Nigeria because, it provides income, creates employment and addresses food insecurity with the provision of low cholesterol animal protein to the majority of African populations (Adebayo and Daramola, 2013). Presently, aquaculture is the fastest growing food production sector in the world (FAO, 2014). However, diseases, especially bacterial infections, can be a significant limiting factor to its continued expansion. This necessitates the intensive use of antimicrobials in the industry (Du and Liu, 2012).

In recent years, a wide variety of chemicals have been used in aquaculture for fish health management. These include disinfectants (hydrogen peroxide and malachite green), anthelmintic (avermectin) and antibiotics (sulfonamide and tetracycline) (Rawn *et al*., 2009). However, the public health concern relating to the use of antibiotics in aquaculture is primarily the development of antibiotics-resistance and immunosuppressant conditions in humans (Cruz *et al*., 2012). It also includes the presence of antibiotic residues in aquaculture products and the environment (Romero-Geraldo and Hernández-Saavedra, 2014).

Hence, in order to ensure sustainable aquacultural development, diseases control strategies must go beyond antibiotics and chemotherapeutics, to new methods gaining recognition for controlling pathogens (Edun and Akinrotimi, 2011), which include the use of probiotics (Suvarna and Boby, 2005). Probiotics, the beneficial live microorganisms, are considered to promote growth, enhance the immunity of fish under stressful environmental conditions, as well as production of antibodies, acid phosphatase, lysozyme and antimicrobial peptides (Abareethan and Amsath, 2015).

Bacillus species, belonging to the phylum Firmicutes, are used in huge amount as human probiotics, and has shown remarkable health benefits (Rane and Markad, 2015). The genus *Bacillus* is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tracts (GIT) of ruminants and humans (Casula and Cutting, 2002; Duc *et al*., 2003). *Bacillus subtilis* is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions (Barbosa *et al*., 2005). Some bacilli strains have been chosen for use in animal nutrition because of their beneficial effects (Busch *et al*., 2004). Consequently, the objective of this study was to examine the effects of *B. subtilis* on growth response, nutrient utilization, biochemical and haematological properties in African cat fish, *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

The catfish, *C. gariepinus* used for this study were obtained from a reputable fish farm in Egbeda, Lagos State, and the experiment was carried out at the Nutrition Unit, Department of Marine Sciences, University of Lagos, Akoka, Lagos, Nigeria.

Bacterial Strain and Sub-culturing

B. subtilis U146A (NCBI accession number: JN255713) previously isolated from *iru* (an alkaline fermented legume seed condiment in Nigeria) (Adewumi *et al*., 2014), and deposited in the culture collection of the Department of Microbiology, University of Lagos, was incorporated into the fish diets. For sub-culturing a pure strain of *B. subtilis* U146A was inoculated into brain heart infusion broth (HiMedia, Mumbai, India) overnight at 37 ^{o}C in incubator shaker at 160 rpm. The broth culture was centrifuged at 8000 rpm for 7 min to make pellets, which were washed twice using phosphate buffer saline (PBS, pH 7.4), and re-suspended in PBS, corresponding to 10^5, 10^7 and 10^9 CFU/ml (Oguntoyinbo and Narbad, 2012).

Feed Formulation

Feed ingredients were sourced from Abattoir, Agege, Lagos, Nigeria. Five experimental diets with crude protein value of 35% and energy content of 3127 kCal were formulated with the following ingredients: fish meal, soybean meal, maize, wheat, dicalcium phosphate (DCP), oil, premix and salt. Measured quantities of all the ingredients were mixed, blended, and passed through a 2 mm die using a local pelletizer. The experimental diets consist of a control, i.e., Diet 1 without antibiotic or probiotics; Diet 2 had antibiotic (oxytetracycline) added to the formulated feed at 20 mg/100 g, while Diets 3, 4 and 5 had *B. subtilis* U146A at the graded levels of 10^5, 10^7, and 10^9 CFU/ml. After pelletizing, the feed was sundried to reduce moisture, after which it was packed in dry plastics. All experimental diets were kept at -20 ^{o}C till when required for the experimental feeding. The feed composition and formulation of the experimental diets are as shown in Table 1.

Table 1: Nutrient composition of experimental diets

			Graded probiotic inclusion levels		
Ingredients (%)	Diet 1 (Control)	Diet 2 (Oxytetracycline)	Diet 3 (10^5)	Diet 4 (10^7)	Diet 5 (10^9)
Fish meal	17.15	17.15	17.15	17.15	17.15
Soybean meal	19.10	19.10	19.10	19.10	19.10
Groundnut cake	19.10	19.10	19.10	19.10	19.10
Maize	20.40	20.40	20.40	20.40	20.40
Noodle waste	20.40	20.40	20.40	20.40	20.40
Palm oil	1.0	1.0	1.0	1.0	1.0
DCP	0.4	0.4	0.4	0.4	0.4
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2
Mineral/vits. premix	1.5	1.5	1.5	1.5	1.5
Salt	0.2	0.2	0.2	0.2	0.2
Probiotics (CFU/mL)	-	-	10^5	10^7	10^9
Oxytetracycline	-	20mg/100g	-	-	-
Total	100	100	100	100	100
Calculated CP (%)	35	35	35	35	35
Cal. energy (kCal/kg)	3127	3127	3127	3127	3127

Vitamin A, 10,000,000 I.U.D.; D3, 2,000,000 I.U.D.; E, 23,000 mg; K3, 2,000 mg; B1, 3000 mg; B2, 6,000 mg; niacin, 50,000 mg; calcium pathonate,10,000 mg; B6, 5000 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.

Experimental Procedure and Feeding Trials

The experiment was carried out in holding plastic tanks (52.5 × 33.5× 21cm^3). One hundred and fifty (150) juvenile cat fish (average weight = 94.33±0.67g) were acclimatized for 2 weeks prior to the commencement of experiment, and were fed *ad libitum* with control feed (35% crude protein and 3127 kCal/kg energy). Ten (10) fish were randomly allocated to five experimental treatments (T_1, T_2, T_3, T_4 and T_5) in three replicates at the end of the adaptation period. Water exchange was done thrice a week with de-chlorinated water supply from a borehole to maintain good water quality. The dissolved oxygen ranged from 4.5 to 6.0 mg/L, while pH and temperature ranged from 6.5 to 7.0, and 26 to 29 ^oC respectively, during the experimental period.

Growth and Nutrient Utilization Parameters

Fish sampling was carried out on a weekly basis by transferring fish from tanks into a weighing bowl. The weights of fish were taken using an electronic weighing balance (2000 ×

0.1 g), and after weighing fish were returned carefully into their respective tanks. The weight data were used to calculate other growth indices using the formulae below:

Mean Weight Gain (MWG) g

MWG = mean final body weight (MFW) – mean initial body weight (MIW)

Percentage weight gain (PWG) %

PWG (%) = 100 $(W_2-W_1)/W_1$

where W_2 = mean final body weight and W_1 = mean initial body weight

Specific growth rate (SGR) = $(Log_e W_2 – Log_e W_1)/(culture\ days)\times100$

where W_2 = final weight, W_1 = initial weight, e = natural logarithm, T = culture days.

Nutrient utilization indices were expressed in terms of Total Feed Intake (TFI), Feed Conversion Ratio (FCR), Protein Intake (PI) and Protein Efficiency Ratio (PER) using the formulae below:

Total Feed Intake (TFI) = Feed intake during experimental period (g)/Number of days

Feed Conversion Ratio (FCR) = Feed intake (dry weight of feed fed in g)/Fish wet weight gain in g

Protein Intake (PI) = Total feed intake/Protein content of feed

Protein Efficiency Ratio (PER) = Mean weight gain/Protein intake

Procedures for Collection of Blood Samples for Haematological and Biochemical Analysis

Haematological Analysis

At the 8th week of feeding, blood samples were collected with the aid of 2 mL syringes from the caudal vasculature of the fish from each treatment group, and emptied into Heparin bottles for haematological analysis at the Department of Medical Laboratory Sciences, Lagos University Teaching Hospital, Idi-Araba, Lagos. Haematological values were measured following standard methods (Blaxhall and Daisley, 1973; Joshi *et al*., 2002). White blood cells (WBC) and red blood cells (RBC) were counted by Neubauer's improved haemocytometer, using Turk's and Hyem's solutions as diluting fluids respectively, packed cell volume (PCV) and haemoglobin (Hb) concentration were analyzed using haematocrit and cyanmethemoglobin methods respectively. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated using the standard method described by Dacie and Lewis (1991). Blood smear were stained using Grunwald-Giemsa stain, for lymphocytes and neutrophils examination (Tavares-Dias *et al*., 1999).

Biochemical Analysis

Blood samples were also collected and emptied into plain bottles for biochemical analysis at the Department of Clinical Chemistry laboratory, Lagos University Teaching Hospital, Idi-

Araba, Lagos. Blood samples were centrifuged at 3000 rpm for 10 min, while the serum obtained were stored at −20 ˚C prior to further analyses.

Serum enzymes: The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to Reitman and Frankel (1957) colometric method using Randox kits, while alkaline phosphatase activity was determined according to phenolphthalein monophosphate method (Babson *et al*., 1966).

Liver antioxidant enzymes: The liver was excised and homogenized in ice-cold 0.25 M sucrose buffer, pH 7.4. The homogenate was centrifuged at 5000 rpm for 15 min at 4 ^{o}C and preserved prior to analysis. Superoxide dismutase (SOD, units/mg protein) activity was determined by its ability to inhibit the auto-oxidation of epinephrine, determined by the increase in absorbance at 480 nm as described by Sun and Zigma (1978). The reaction mixture (3 mL) contained 2.95 mL of 0.05 M sodium carbonate buffer (pH 10.2), 0.02 mL of the blood sample and 0.03 mL of epinephrine in 0.005 N HCl. Catalase (CAT, µmol/mg protein) activity was determined according to Sinha (1972), wherein dichromatic acetic acid, following heating in the presence of H_2O_2, undergoes reduction to chromic acetate, with perchloric acid being formed; this was analyzed spectrophotometrically at 590 nm. The activity of glutathione (GSH, units/mg protein) was determined in the tissue homogenates using Ellman's reagent, 5-5-dithio-bis (2-nitrobenzoic acid) (DTNB) as a colouring reagent (Sedlak and Lindsay, 1968).

Statistical Analysis

Data were analyzed with one-way ANOVA, and means were compared using Duncan Multiple Range Test (Duncan, 1955) at significant level of 0.05. All computations were performed using statistical package IBM 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The results of growth and nutrient utilization parameters of *C. gariepinus* juveniles fed with experimental diets are as shown in Table 2. The results showed that the highest significant (p<0.05) mean weight gain was achieved with fish fed diet 5 (116.67±5.70), followed by diet 4 (103.17±3.79), diet 2 (103.00±4.16), diet 3 (96.42±6.43), and the least value by the group fed diet 1 (89.00±0.58). In addition, the highest percentage weight gain (PWG) was recorded by diet 5 (133.62±7.47%). This was significantly different (p<0.05) from other experimental groups, and the least value (94.35±0.91) was recorded for the control group. The highest values for total feed intake (154.63±7.19) and daily feed intake (3.16±0.15) were recorded among the groups of fish fed diet 2 (oxytetracycline); these values were significantly different (p<0.05) from other groups of fish fed probiotic diets. The best significant value (p<0.05) for feed conversion ratio (FCR) was recorded with diet 5 (1.17±0.04), while the least (1.60±0.06)

was recorded for the control diet. The PER for diets 1 and 5 recorded the lowest (2.54±0.02) and highest (3.27±0.18) values respectively, and were also significantly different (p<0.05) from other diets. No significant variation was observed in groups fed diets 2, 3 and 4 whereas, diets 1 and 2 fed groups differed significantly (p<0.05). Furthermore, no significant difference (p>0.05) was recorded in the values of protein intake (PI), with the exception of diets 1 and 2. The highest PI (54.12±2.52) was recorded by the group fed diet 2, while the group fed diet 5 recorded the least value (46.28±1.32). The groups of fish fed diet 2 (oxytetracycline) and diet 5 recorded the highest (154.63±7.19) and lowest (132.23±3.77) values for total feed intake (TFI), while the groups of fish fed with *B. subtilis* differed significantly (p< 0.05) from diets 1 and 2.

Table 2: Growth and nutrient utilization parameters of *C. gariepinus* juveniles fed with experimental diets, containing probiotic *Bacillus subtilis* U146A

Parameters	Diet 1 (Control)	Diet 2 (Oxytetracyline)	Diet 3 (10^5)	Diet 4 (10^7)	Diet 5 (10^9)
MFW g	183.33±0.88^{a}	197.00±5.03^{ab}	189.75±6.32^{a}	199.67±3.60^{ab}	212.33±5.57^{b}
MIW g	94.33±0.67	94.00±2.00	93.33±0.99	96.50±0.89	95.67±0.33
MWG g	89.00±0.58^{a}	103.00±4.16^{ab}	96.42±6.43^{a}	103.17±3.79^{ab}	116.67±5.70^{b}
PWG %	94.35±0.91^{a}	109.63±4.40^{ab}	103.31±7.17^{ab}	106.91±4.10^{ab}	133.62±7.47^{b}
SGR %/day	1.36±0.01^{a}	1.51±0.04^{ab}	1.44±0.07^{ab}	1.42±0.04^{ab}	1.58±0.07^{b}
TFI g	142.13±5.09^{ab}	154.63±7.19^{b}	133.72±2.74^{a}	136.23±6.00^{a}	132.23±3.77^{a}
DFI g/day	2.90±0.10^{ab}	3.16±0.15^{b}	2.73±0.06^{a}	2.78±0.12^{a}	2.70±0.08^{a}
FCR	1.60±0.06^{c}	1.50±0.03^{bc}	1.41±0.09^{bc}	1.37±0.06^{ab}	1.17±0.04^{a}
PER	2.54±0.02^{a}	2.94±0.12^{ab}	2.75±0.18^{ab}	2.86±0.11^{ab}	3.27±0.18^{b}
PI	49.75±1.78^{ab}	54.12±2.52^{b}	46.80±0.96^{a}	47.68±2.10^{a}	46.28±1.32^{a}

Values on the same row with different superscripts are significantly different (p<0.05). Mean final body weight (MFW), mean initial body weight (MIW), mean weight gain (MWG), percentage weight gain (PWG), specific growth rate (SGR), total feed intake (TFI), daily feed intake (DFI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein intake (PI).

The effects of the *B. subtilis* U146A probiotic on the blood parameters of experimental fish are recorded in Table 3. Although, the values of the haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV) decreased with the increasing inclusion of graded levels of probiotic and oxytetracycline. However, no significant difference (p>0.05) was recorded among dietary treatments. Equally, there was no significant difference (p>0.05) across all experimental groups in the following parameters; mean corpuscular haemoglobin concentration (MCHC), neutrophils (NEUT), lymphocytes (LYM), monocytes (MONO) except, mean corpuscular haemoglobin (MCH) in which diets 2 and 3 significantly different (p<0.05) from other experimental diets (Table 3).

Table 3: Haematological parameters of *C. gariepinus* juveniles fed with experimental diets, containing probiotic *Bacillus subtilis* U146A

Parameters	Diet 1 (Control)	Diet 2 (Oxytetracyline)	Diet 3 (10^5)	Diet 4 (10^7)	Diet 5 (10^9)
WBC X(10^9/L)	63.13±9.08	53.83±9.24	50.00±4.28	41.17±8.44	53.53±4.80
PCV (%)	39.23±2.63	32.27±1.18	33.35±1.58	28.07±5.39	35.40±1.97
Hb (g/L)	15.10±0.91	13.10±0.61	12.65±0.56	10.78±1.98	13.63±0.53
RBC X(10^9/L)	2.53±0.17	2.32±0.18	2.09±0.11	1.83±0.35	2.34±0.11
MCH(Pg)	155.13±3.40^{ab}	139.57±5.55^{a}	160.58±6.05^{b}	153.43±6.68^{ab}	151.22±4.01^{ab}
MCHC (g/L)	59.73±0.60	56.87±1.68	60.97±1.22	60.08±1.55	58.45±1.19
MONO (%)	38.53±0.95	40.77±0.44	38.10±0.93	39.63±2.43	38.72±1.04
LYM (%)	0.27±0.03	0.20±0.06	0.65±0.15	1.13±0.46	0.28±0.06
NEUT (%)	61.10±0.05	59.10±0.09	61.25±0.37	59.24±1.83	61.00±0.08

Values on the same row with different superscripts are significantly different (p<0.05) from each other. White blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophils (NEUT), lymphocytes (LYM), monocytes (MONO).

The biochemical characteristics of *C. gariepinus* fed diets with different levels of *B. subtilis* U146A and antibiotic are shown in Table 4. There was no significant difference (p>0.05) in the values recorded for AST, ALP and GSH across diets. Similarly, no significant difference (p>0.05) was found in values of ALT, with the exception of control (35.67±18.17), which differed significantly (p<0.05) across experimental diets. Likewise, SOD values were significantly high (p<0.05) in fish fed diet 4 (158.44±1.88) and antibiotic diet (157.81±5.73), which showed remarkable increase over other dietary groups. Furthermore, CAT values were significantly high (p<0.05) in fish fed control diet (655.22±89.48) and antibiotic diet (749.28±3.26), which also showed remarkable increase over other groups (Table 4).

Table 4: Biochemical parameters of *C. gariepinus* juveniles fed with experimental diets, containing probiotic *Bacillus subtilis* U146A

Parameters	Diet 1 (Control)	Diet 2 (Oxytetracyline)	Diet 3 (10^5)	Diet 4 (10^7)	Diet 5 (10^9)
AST (U/L)	65.67±16.76	63.33±14.08	60.83±12.48	58.00±5.26	59.20±4.53
ALT (U/L)	35.67±18.17^b	18.00±1.53^a	18.50±1.18^a	14.80±2.37^a	15.40±1.17^a
ALP (U/L)	12.00±0.58	16.33±1.86	12.33±1.67	12.40±0.51	12.40±1.60
GSH (units/mg protein)	49.92±11.02	44.08±1.31	41.54±16.04	42.17±2.67	50.11±4.56
SOD (units/mg protein)	145.47±3.18^b	157.81±5.73^c	139.22±4.67^b	158.44±1.88^c	82.70±1.66^a
CAT (µmol/mg protein)	655.22±89.48^b	749.28±3.26^b	522.80±113.79^a	453.62±67.08^a	391.16±21.81^a

Values on the same row with different superscripts are significantly different (p<0.05) from each other. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH).

DISCUSSION

Probiotics, which are live microorganisms that confer health benefits on the host, have been used in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds (Tekinay and Davies, 2001**)**. In this study, the growth parameters of the experimental fish were significantly enhanced by the supplementation of the probiotic microorganism (*B. subtilis*) at all the inclusion rate, especially at the highest level. In addition, the group of fish fed diet 5 recorded the lowest total feed intake with highest mean weight gain. These results shows the beneficial effects of *B. subtilis*, which enhances gut functions, thereby helping the activities of endogenous enzymes like protease, whose main function is to digest protein into components required for tissue growth. This was corroborated by the work of Hauville *et al*. (2016), who reported positive results when they fed a mixture of commercial *Bacillus* to Florida pompano and common snook larvae during their early larval stages, to determine the effect on growth and digestive enzyme activities. Several other studies have demonstrated the positive effects of *Lactobacillus*

species on the growth response of gilthead sea bream (Suzer *et al.*, 2008), African catfish (Al-Dohail *et al.*, 2009), Persian sturgeon and beluga fry (Sarker *et al.*, 2010).

The possible reason for the improved growth performance of *C. gariepinus* after feeding with probiotic diets might be due to improved gut functions and feed efficiency of diet (Al-Dohail *et al.*, 2009), which ultimately stimulated the appetite of fish (Irianto and Austin, 2002). The enhanced growth could be due to the ability of *B. subtilis* to stimulate appetite and improve the absorption of nutrients (Wang *et al*., 2008). Other microorganisms such as *Agrobacterium* sp., *Pseudomonas* sp., *Brevibacterium* sp., *Microbacterium* sp. and *Staphylococcus* sp. have also been documented as having the potential to contribute to nutritional processes (Lara-Flores, 2011). Similar observations have been reported for the microbial flora of adult penaeid shrimp (*Penaeus chinensis*), where a complement of enzymes exists for digestion and synthesis of compounds that are assimilated by the animal (Mohammed, 2015). Also, there were reports that *B. subtilis* can improve the growth, survival and immune system of *Oreochromis niloticus* (Aly *et al*., 2008) and shrimp (*Penaeus monodon*) (Rengpipat *et al*., 2000).

Haematological parameters, especially PCV, total and differential leukocyte counts in the blood, provide an indication of the health status of the fish (Hrubec *et al*., 2000). Equally, blood characteristics of most fish have been studied to establish normal value range, and deviation from it may indicate a disruption in the physiological process of fish (Rainza-Paiva *et al*., 2000; Joshi *et al*., 2002). Consequently, the mean values obtained in this study were within the normal ranges recommended for *C. gariepinus* and also exhibited that its wellbeing is in good condition (Erhunmwunse and Ainerua, 2013).

Similarly, *O. niloticus* fed diet supplemented with *B. subtilis* (Soltan and El-Laithy, 2008) and *Pediococcus acidilactici* (Ferguson *et al.*, 2010) showed some variation, but no significant difference in Hb and PCV contents among control and the other experimental fish groups fed diet enriched with probiotics. On the contrary, Abd El-Rhman *et al*. (2009), reported significant effects on haematological parameters when probiotics were applied in Tilapia diet. The reason for this may be due to the different genera of probiotic bacteria used for feed formulations. *B. subtilis* was included in fish feed meal in this study, while *Micrococcus luteus* and *Pseudomonas* species were employed in the study conducted by Abd El-Rhman *et al*. (2009).

Modulation of immune system is one of the numerous benefits attributed to probiotics (Nayak, 2010). *B. subtilis* cells as probiotics have been reported to shape the immune system by their physiological action in the intestines, and upon colonizing the gut they trigger an immune response because the intestinal cells can produce a series of immunoregulatory molecules when stimulated by bacteria (Corcionivoschi *et al*., 2010). This was the case in the present study.

The results obtained on the effect of probiotics on biochemical indices showed that the control diet had the highest values for ALT and AST. In addition, the value of ALT for control group was significantly higher than other diets. This has revealed that the probiotic *B. subtilis* has positively modulated the above parameters, resulting in the improved health status of the fish. This was in agreement with the work of Adorian *et al*. (2019) who reported that liver enzymes (AST, ALT and ALP) were lower in fish fed diet supplemented with 1×10^6 CFU g^{-1} probiotic *Bacillus* compared with the control group.

Antioxidant enzymes are crucial in the effort to counteract oxidative stress caused by toxicants once the supply of other antioxidant compounds is depleted. These enzymes, which remove peroxides, and superoxide radicals including SOD, catalase and GSH are of essence in oxidative stress to deal with free radicals causing several disturbances (Saglam *et al*., 2014). Catalase degrades the hydrogen peroxide produced by the dismutation of superoxide ion by SOD during oxidative stress. In this study, the effect of *B. subtilis* has greatly suppressed the activities of antioxidant enzymes, particularly at the highest supplementation with probiotic, the values of SOD and CAT were greatly reduced. This further buttresses the fact that the group of fish fed probiotic were not under stress compared to the control and oxytetracycline groups. According to Han *et al*. (2016), SOD concentration increases with the intensity of stress, but the activity of catalase and GSH can vary depending upon the type of stress. This was further corroborated by the work of Shaheen *et al*. (2014) who reported that commercial feed supplemented with probiotic resulted in lower expression levels of glutathione peroxidase (GPx), SOD and cytochrome c oxidase subunit 1 (COX1), compared to the control feed in two yellow perch. They attributed the differences in gene expression to be due to the presence of probiotic, assuming a possible involvement in the modulation of the antioxidant system in the fish. Therefore, from this study we could conclude that among probiotic beneficial effects, is to provide protection against oxidative stress, and the ability to decline the risk of accumulation of reactive oxygen metabolites, which are harmful to the host.

CONCLUSION

The results obtained from this study show that *B. subtilis* modulates the gut microbes, thereby enhancing nutrients absorption and consequently improves the weight gain, at 10^9 CFU/mL level in the diet of *C. gariepinus* for a sustainable high productivity in African mud cat fish farming.

ACKNOWLEDGEMENTS

Project involving the molecular characterization and identification of *B. subtilis* U146A isolated from *iru* (a traditional alkaline fermented legume seed condiment in Nigeria), was supported by funding through the award of a DBT-TWAS sandwich postgraduate fellowship to one of the authors, Gbenga Adedeji Adewumi, which facilitated his research visit to the Institute of Bioresources and Sustainable Development (IBSD), Imphal, India.

REFERENCES

Abareethan, M. and Amsath, A. (2015). "Characterization and evaluation of probiotic fish feed". ***International Journal of Pure and Applied Zoology***. 3(2): 148-153.

Abd El-Rhman, A.M.A., Khattab, Y.A. and Shalaby, A.M. (2009). "*Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus"*. ***Fish & Shellfish Immunology***. 27(2): 175-180.

Adebayo, O.O. and Daramola, O.A. (2013). "Economic analysis of cat fish (*Clarias gariepinus)* production in Ibadan Metropolis". ***Journal of Agriculture and Food Sciences***. 1(7): 128-134.

Adewumi, G.A., Oguntoyinbo, F.A., Romi, W., Singh, T.A. and Jeyaram, K. (2014). "Genome sub-typing of autochthonous *Bacillus* species isolated from *iru*, a fermented *Parkia biglobosa* seeds". ***Food Biotechnology***. 28: 250-268.

Adorian, T.J., Jamali, H., Farsani, H.G., Daryishi, P., Hasanpour, S., Bagheri, T. and Roozbehfar, R. (2019). "Effects of probiotic bacteria *Bacillus* on growth performance, digestive enzyme activity, and haematological parameters of Asian sea bass, *Lates calcarifer* (Bloch)". ***Probiotics and Antimicrobial Proteins***. 11: 248-255.

Al-Dohail, M.A., Hashim, R. and Aliyu-Paiko, M. (2009). "Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African catfish (*Clarias gariepinus*, Burchell 1822) fingerling". ***Aquaculture Research***. 40(14): 1642-1652.

Aly, S.M., Mohamed, M.F. and John, G. (2008). "Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*)". ***Aquaculture Research.*** 39(6): 647-656.

Babson, A.L., Greeley, S.J., Coleman, C.M. and Philips, G.E. (1966). "Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase". ***Clinical Chemistry***. 12: 482-490.

Barbosa, T.M., Serra, C.R., La Ragione, R.M., Woodward, M.J. and Henriques, A.O. (2005). "Screening for *Bacillus* isolates in the broiler gastrointestinal tract". ***Applied and Environmental Microbiology.*** 71: 968-978.

Blaxhall, P.C. and Daisley, K.W. (1973). "Routine haematological methods for use with fish blood". ***Journal of Fish Biology***. 5: 771-781.

Busch, A., Herrmann, H.H., Kühn, I., Simon, O., Struck, J., and Süphke, E. (2004). "Probiotics in animal nutrition". *Arbeitsgemeinschaft für Wirkstoffe in der Tierernährung* eV.*Agrimedia,* Bonn, Germany: Agrimedia GmbH.

Casula, G. and Cutting, S.M. (2002). "*Bacillus* probiotics: spore germination in the gastrointestinal tract". ***Applied and Environmental Microbiology*** 68: 2344-2352.

Corcionivoschi, N., Drinceanu, D., Pop, I.M., Stack, D., Ştef, L., Julean, C. and Bourke, B. (2010)."The effect of probiotics on animal health". ***Scientific Papers: Animal Science and Biotechnologies***. 43(1): 35-41.

Cruz, M.P., Ibáñez, A.I., Monroy Hermosillo, O.A. and Saad, H.C.R. (2012). "Use of probiotics in aquaculture". ***International Scholarly Research Network Microbiology***.1-14. Article ID 916845, DOI: 10.5402/2012/916845.

Dacie, S.I.V. and Lewis, S.M. (1991). Practical haematology (7th edition) J and A Churchill Ltd. Livingston, London, Melborne and New York, 67pp.

Duc, L.H., Hong, H.A. and Cutting, S.M. (2003). "Germination of the spore in the gastrointestinal tract provides a novel route for heterologous antigen presentation". ***Vaccine.*** 21: 4215-4224.

Du, L. and Liu, W. (2012). "Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review". ***Agronomy for Sustainable Development***. 32: 309-327.

Duncan, D. B. (1955). "Multiple range and multiple F test". ***Biometrics***. 11: 1-42.

Edun, O.M. and Akinrotimi, O. (2011). "The use of probiotics in aquaculture. ***Nigerian Journal of Biotechnology***. 22: 34-39.

Erhunmwunse, N. and Ainerua, M. (2013). "Characterization of some blood parameters of African catfish (*Clarias gariepinus*)". ***American-Eurasian Journal of Toxicological Sciences.*** 5: 72-76.

FAO (2014). The State of World Fisheries and Aquaculture. Available at: http://www.fao.org/3/a-i3720e.pdf.

Ferguson, H.W., Christian, M.D., Hay, S., Nicolson, J., Sutherland, D. and Crumlish, M. (2010). "Jellyfish as vectors of bacterial disease for farmed salmon (*Salmo salar*)". ***Journal of Veterinary Diagnostic Investigation.*** 22(3): 376-382.

Han, C.Y., Zeng, Q.M. and Sun, Z.T. (2016). Gene expression of antioxidant enzymes in hybrid tilapia, *Oreochromis niloticus* X *Oreochromis aureus* under acute pH stress. ***Journal of the World Aquaculture Society.*** 47(2): 260-267.

Hauville, M.R., Zambonino-Infante, J.L., Gordon Bell, J., Migaud, H. and Main, K.L. (2016). "Effects of a mix of *Bacillus* sp. as a potential probiotic for Florida pompano, common snook and red drum larvae performances and digestive enzyme activities". ***Aquaculture Nutrition.*** 22(1): 51-60.

Hrubec, T.C., Cardinale, J.L. and Smith, S.A. (2000). "Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*)". ***Veterinary Clinical Pathology.*** 29(1): 7-12.

Irianto, A. and Austin, B. (2002). "Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum)". ***Journal of Fish Diseases***. 25(6): 333-342.

Joshi, P.K. Bose, M. and Harish, D. (2002). “Changes in certain haematological parameters in siluroid catfish, *Clarias batrachus* (Linn) exposed to cadium chloride”. ***Pollution Resources***. 21(2): 129-131.

Lara-Flores, M. (2011). “The use of probiotic in aquaculture: an overview”. ***International Research Journal of Microbiology***. 2(12): 471-478.

Mohammed, G.S. (2015). “Use of probiotics as biological control agents in Aquaculture For sustainable development”. ***International Journal of Food, Agriculture and Veterinary Sciences***. 5(1): 112-119.

Nayak, S.K. (2010). “Probiotics and immunity: a fish perspective”. ***Fish Shellfish Immunology***. 29(1): 2-14.

Oguntoyinbo, F.A. and Narbad, A. (2012). “Molecular characterization of lactic acid bacteria and in situ amylase expression during traditional fermentation of cereal foods”. ***Food Microbiology***. 31: 254-262.

Rainza-Paiva, M.J.T., Ishikawa, C.M., Das Eiras, A.A. and Felizardo, N.N. (2000) “Haematolocal analysis of chara, *Pseudoplatystoma fasciatum* in captivity”. In: Aqua 2000: Responsible Aquaculture in the New Millenium, Nice, France, 2-6 May 2000. Special Publication 28. *European Aquaculture Society*. 590 pp.

Rane, M., and Markad, A. (2015). “Effects of probiotic on the growth and survival of zebra fish (*Danio rerio*)”. ***International Journal of Scientifical Research***. 4(3): 1839-1841.

Rawn, D.F.K., Breakell, K., Verigin, V., Nicolidakis, H., Sit, D., Feeley, M., and Ryan, J.J. (2009). “Persistent organic pollutants in fish oil supplements on the Canadian market: Polychlorinated dibenzo-p-dioxins, dibenzofurans, and polybrominated diphenyl ethers”. ***Journal of Food Science***. 74(4): 1-15.

Reitman, S. and Frankel, S. (1957). “Glutamic – pyruvate transaminase assay by colorimetric method”. ***American Journal of Clinical Pathology***. 28: 56-58.

Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S. and Menasaveta, P. (2000). “Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by aprobiont bacterium (*Bacillus* S11)”. ***Aquaculture.*** 191: 271–288.

Romero-Geraldo, R.D.J. and Hernández-Saavedra, N.Y. (2014). “Stress gene expression in *Crassostrea gigas* (Thunberg, 1793) in response to experimental exposure to the toxic dinoflagellate *Prorocentrum lima* (Ehrenberg) Dodge, 1975”. ***Aquaculture Research.*** 45(9): 1512-1522.

Saglam, D., Atli, G., Dogan, Z., Baysoy, E., Gurler, C., Eroglu, A. and Canli, M. (2014). “Response of the antioxidant system of freshwater fish (*Oreochromis niloticus*) exposed to metals (Cd, Cu) in differing hardness”. ***Turkish Journal of Fisheries and Aquatic Sciences.*** 14: 43-52.

Sarker, S.K., Park, S.R., Kim, G.M. and Yang, C.J. (2010). "Hamcho (*Salicornia herbacea*) with probiotics as alternative to antibiotic for broiler production". ***Journal of Medicinal Plants Research.*** 4(5): 415-420.

Sedlak, J. and Lindsay, R. H. (1968). Estimation of total protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. ***Analytical Biochemistry***. 25: 1192-1205.

Shaheen, A.A., Eissa, N., Abou-ElGheit, E.N., Yao, H. and Wang, H.P. (2014). Probiotic effect on molecular antioxidant profiles in yellow perch, *Perca flavescens*. ***Global Journal of Fisheries and Aquaculture Researches***. 1(2): 16-29.

Sinha, A. K. (1972). "Colorimetric assay of catalase". ***Analytical Biochemistry***. 89: 95-10.

Soltan, M.A. and El-Laithy, S.M.M. (2008). "Effect of probiotics and some spices as feed additives on the performance and behaviour of Nile tilapia, *Oreochromis niloticus"*. ***Egyptian Journal of Aquatic Biology and Fisheries.*** 12(2): 63-80.

Sun, M. and Zigma, S. (1978). "An improved spectrophotometer assay of superoxide dismutase based on epinephrine antioxidation". ***Analytical Biochemistry***. 90: 81-89.

Suvarna, V.C. and Boby, V.U. (2005). "Probiotics in human health: A current assessment". ***Current Science***. 88(11): 1744-1748.

Suzer, C., Çoban, D., Kamaci, H.O., Saka, Ş., Firat, K., Otgucuoğlu, Ö. and Küçüksari, H. (2008). "*Lactobacillus* spp. bacteria as probiotics in Gilthead Sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities". ***Aquaculture.*** 280(1): 140-145.

Tavares-Dias, M., Afonso, G.E., Oliveira, S.R., Marcon, J.L. and Egami, M.I. (1999). "Comparative study on haematological parameters of matrinxa Brycon amazonicus". ***Acta Amazonica.*** 38(4): 799-806.

Tekinay, A.A. and Davis, S.J. (2001). "Dietary carbohydrate level influencing feed intake, nutrient utilisation and plasma glucose concentration in Rainbow Trout". ***Turkish Journal of Veterinary and Animal Sciences***. 25(5): 657-666.

Wang, Y.B., Li, J.R. and Lin, J. (2008). Probiotics in aquaculture: challenges and outlook. ***Aquaculture.*** 281(1-4): 1-4.