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Zootechnical Performance and Haematology of African Catfish *Clarias gariepinus* (Burchell, 1822) fed *Lactobacillus pentosus* supplemented diets

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Abstract

The zootechnical performance and haematological profile of African catfish *Clarias gariepinus* juveniles, fed with commercial probiotic, *Lactobacillus pentosus* supplemented diets were assessed in this study which lasted for 56 days. Six experimental diets were formulated, at different inclusion levels of the probiotic *L. pentosus* of 0, 10^2, 10^4, 10^6, 10^8 and 10^{10} CFU and denoted as T1 (control), T2, T3, T4, T5, and T6 respectively. After the feeding trial, zootechnical and blood parameters were evaluated. Results showed that zootechnical parameters were significantly ($P < 0.05$) enhanced with increasing supplementation levels of *L. pentosus*. Growth and nutrient utilization indices like weight gain, specific growth rate, protein efficiency and feed conversion ratio responded with curvilinear trends. There were improvements in these parameters as the level of *L. pentosus* was increased to 10^8 CFU in the diets, followed by adverse responses when the *L. pentosus* level was increased to 10^{10} CFU of the diet. A second degree polynomial regression model depict a strong relationship between SGR and *L. pentosus* supplementation in fish diet. There were significant differences ($P < 0.05$) in the haematological parameters of fish fed the experimental diets. This study also revealed that PCV and Red Blood Cells increased with increasing levels of *L. pentosus* in the diet of *C. gariepinus* and peaked at 10^8 CFU of the probiotic supplementation in T5. However the WBC decreased almost linearly as the level of *L. pentosus* in the diet was increased. The best supplementation level of *L. pentosus* was 10^8 CFU in T5. From the above deductions, this study confirmed the nutritional and health benefits of probiotic *Lactobacillus pentosus* in the practical diet of African catfish, *C. gariepinus.*

1. INTRODUCTION

Globally, the demand for fish and fish products is increasing as a result of increasing population and public awareness of the health benefits of consuming fish [1, 2]. Fish is one of the cheapest and direct sources of protein and micro nutrients for millions of people in Africa and Nigeria in particular [3]. There is a wide gap between fish demand and supply in Africa and aquaculture has been identified as the best option to bridge the wide gap between fish demand and domestic production in most countries of the world especially the sub Saharan Africa [2]. The growth of fish is an important parameter to be put into consideration in aquaculture, and good fish growth is essential in getting a good yield. For growth to take place, it is important that the formulated fish feed is eaten and utilized adequately, so as to ensure that it has a good feed conversion ratio (FCR). A good culture medium can determine the fish's growth performance; this can assist in assessing the feed acceptability, digestion, and immune development [4].

The need for sustainable aquaculture has promoted research into the use of probiotics on aquatic organisms. The initial interest on the use of probiotics was focused on their use as growth promoters and to improve the health of animals [1, 5]. Probiotics have been used in aquaculture to increase the growth of cultivated species. Probiotic are microorganisms that have the ability to colonize the gastrointestinal tract of fish when administered over a long period of time because they have a higher multiplication rate than the rate of expulsion, so as probiotics are constantly added to fish cultures, they adhere to the intestinal mucosa of the fish, developing and exercising their multiple benefits [6].

Antibiotics have been used for a long time in aquaculture to prevent diseases; however, this caused problems such as the presence of antibiotic residue in the animal tissue, the generation of bacterial resistance mechanisms, as well as imbalance in the gastrointestinal microbiota of aquatic species, which affected their health [7]. Currently, consumers demand natural products free of synthetic additives such as antibiotics [5]. Viable probiotics administered to *Oreochromis niloticus*, increased non-specific immune response, determined by parameters such as lysozymes activity, neutrophile migration and bactericidal activity, which improved the resistance of fish to infection [8]. Furthermore, probiotics have a beneficial effect on the digestive processes of aquatic animals because probiotic strains synthesize extra cellular enzymes such as proteases, amylases, and lipases as well as provide growth factors such as vitamins, fatty acid and amino acids [6]. Therefore nutrients are absorbed more efficiently when the feed is supplemented with probiotics [9].

Probiotics are used as dietary supplements in aquaculture and their roles in intestinal microbial balance, growth, nutrition, health status and resistance against infectious agents are already established [10]. Probiotics may prevent potential pathogens from colonizing the gut by production of antimicrobial compounds [6]. Developments in fish nutrition and health studies showed that a balance must be achieved between optimal fish production and maintaining fish health, because of the vital interactions that exist between nutrients and health [11]. Therefore, a balance needs to be achieved between the use of probiotics as nutritional supplements and maximum level of inclusion that can provide optimal growth without compromising the health of the fish. This will help aquaculture to increase the availability of fish therefore reducing the protein shortage currently witnessed by the growing world population [2].

The African catfish is the most important fish species cultured in Nigeria, this species has shown considerably potential as a fish for intensive aquaculture [12]. This fish grows rapidly, are resistant to disease and stress, sturdy and are highly productive even in polyculture with many other fish species like Nile Tilapia, *Oreochromis niloticus* [1]. In African catfish farming, feed represents 60 to 70 percent of the major cost of intensive catfish production and it is one of the important factors that influence the ability of fish to attain its genetic potential for growth and maintain proper health [12]. Despite, many kinds of literature available on the nutraceuticals efficacy of probiotics on fish nutrition and health status[6, 5], research concerning the effects of commercial probiotics, *Lactobacillus pentosus* on blood and zootechnical parameters in tropical fish species are yet scarce. This work focuses on accessing the use of commercial probiotics, *L. pentosus*, in improving the nutritional and health performance of the African catfish, *Clarias gariepinus*.

2. MATERIALS AND METHOD

2.1 Experimental fish and feeding trial

C. gariepinus juveniles were obtained from the Hatchery unit of the Department of Fisheries and Aquaculture, Federal University of Technology Akure, prior to the feeding trial. Fish were graded by size and groups of 15 fish of 32.82 ± 0.10 g per replicate and the experiment was in triplicate. Fish were stocked into glass tanks of 60cm ×45cm×45cm dimension. A commercial diet, Nutreco

® (40% crude protein) was fed to all fish during a 2- week conditioning period. Each experimental diet was fed to six replicate groups of fish for 56days. All groups were fed at the same fixed rate (initially 5% of body weight per day) which was adjusted each week. Fish were fed by 0900-1000 and 1700-1800h GMT, for 7 days and growth was monitored weekly by batch weighing of fish from each tank.

2.2 Experimental diet and design

Six isonitrogenous diets containing 40% crude protein were formulated for post juvenile African Catfish. All dietary ingredients were milled into small particle size. Lyophilized *Lactobacillus pentosus* (Lennux company, USA) was used as probiotics and was supplemented at 0.00, 10^2cfu, 10^4cfu, 10^6cfu, 10^8cfu and 10^{10}cfu denoted as T1 (control), T2, T3, T4, T5, and T6 respectively (Table 1). The dry particle was thoroughly mixed by adding hot water until a constant dough was achieved. The dough was pelleted using a Hobart pelleting machine (Hobart Model 200, CA, USA) with a 2.0mm die. After pelleting, the diets were sun dried for a week to avoid mould formation and afterwards broken into small sizes and packed in air tight containers and labeled appropriately. Starch was used as binder. A sample of the diet was taken for proximate analysis according to the method of AOAC [13]. The experiments consisted of six treatments, with each representing different supplementation levels of *L. pentosus*.

Table 1: Composition of the experimental diet in g/100g dry matter containing graded levels of *lactobacillus pentosus* supplementation for *Clarias gariepinus*

Ingredients	**T1(Control)**	**T2**	**T3**	**T4**	**T5**	**T6**
Fishmeal(66%)	26.00	26.00	26.00	26.00	26.00	26.00
Soyabean meal (45%)	27.10	27.10	27.10	27.10	27.10	27.10
Groundnut cake (48%)	24.40	24.40	24.40	24.40	24.40	24.40
Maize	7.50	7.50	7.50	7.50	7.50	7.50
Rice bran	7.00	7.00	7.00	7.00	7.00	7.00
Vitamin/mineral premix	3.00	3.00	3.00	3.00	3.00	3.00
Vegetable oil	3.00	3.00	3.00	3.00	3.00	3.00
Starch	2.00	2.00	2.00	2.00	2.00	2.00
Lactobacillus pentosus(cfu)	0.00	10^2	10^4	10^6	10^8	10^{10}

Proximate composition of experimental diets used in feeding African catfish, *Clarias gariepinus* (DM)

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Moisture	10.33	10.16	10.53	10.12	9.68	10.45
Ash	10.81	11.01	10.71^b	10.08	10.08	11.11
Lipid	20.73	20.00	21.00	20.85	20.40	19.98
Protein	39.77	39.17	39.45	39.65	39.60	39.50
Fibre	4.43	4.32	4.35	4.16	4.35	4.50
NFE	13.95	15.30	13.97	15.15	14.85	14.47

NFE, Nitrogen Free Extract (% NFE = 100- %(crude protein + moisture + ether extract + ash + crude fibre).

Vitamins and minerals supplied by Vitamix fish premix: 50,000,000 I.U, vitamin D3, 1,600,000 I.U, vitamin E 15,000, thiamine, 2000 mg, riboflavin, 7500 mg, vitamin B6, 3000 mg, vitamin B12,20 mg, vitamin K, 2000 mg, vitamin C,100,000 mg, nicotinic acid, 10,000 mg, folic acid, 600 mg, biotin, 0.5 mg, BHT, 125,000 mg, manganese, 100,000 mg, iron, 100,000 mg, zinc, 40,000 mg, copper, 5000 mg, iodine, 500 mg, cobalt, 250 mg, selenate, 125 mg, zinc bacitracin, 15,000 mg, chloride, 20,000 mg.

1.4 Water quality assessment

The physical assessment of culture water was carried out weekly and included: temperature, pH, and dissolved oxygen (DO).The water was maintained at 27 - 30 ^{o}C, dissolved oxygen at 6.5-8.3 mg/L and pH 6.0 - 8.5.Dissolved oxygen was monitored using HANNA 98103SE (HANNA instruments, Rhode Island). Temperature and pH were monitored using YSI-IODO 700 Digital probe (IFI Olsztyn, Poland).

1.5 Fish blood collection and analysis

At the end of the feeding trial, fish from each treatment were removed from culture tanks for blood analysis according to Svobodova et al (1991). Five mililitres (5ml) of blood was collected from the fish by cardiac puncture using different 5ml disposable heparinized syringes, with ethylene diamine tetracetic acid (EDTA tubes) EDTA in the tubes served as anticoagulant. The sample was used to determine the number of red blood cells (RBC) and white blood cells (WBC) by means of a haemocytometer slide (Improved Neubauer type) at a magnification of x400. Haematocrit (Hct) was determined by the microhematocrit method. Haemoglobin (Hb) concentration was measured by the cyanohaemoglobin method. Whole blood was mixed with 5 ml of Drabkin's solution (Sigma-Aldrich) in a test tube before allowing to stand for at least 15 min at room temperature. The haemoglobin concentration was calculated from a curve prepared from known standards. Differential white blood cell count was performed to quantify the circulatory levels of lymphocytes, granulocytes and monocytes. One drop of blood was smeared on each slide. Slides were air-dried at room temperature, fixed in 95% methanol and stained with Giemsa stain (Sigma chemical Co. Ltd, Poole, UK) for 20 min and mounted. Lymphocytes, granulocytes and monocytes were identified following the descriptions of [14].

1.6 Evaluation of *zootechnical* parameters

Fish zootechnical performance during the experiment was based on the productivity indices on growth performance and nutrient utilization efficiencies as described by [15].
Mean weight gain: this was calculated as the difference between the final mean weight and the initial mean weight of the experimental fish.
Mean weight gain (g) = Final mean weight – Initial mean weight
Specific growth rate: was calculated from the relationship between the differences in the Log weight of fish within the experimental period as follows;
SGR (%)= (Log_e final weight - Log_e initial weight) / Feeding days X 100
% Weight gain = Final weight – Initial weight / Initial weight X 100
Feed Conversion Ratio (FCR)
FCR = Total feed consumed by fish / Weight gain by fish
Protein Efficiency Ratio (PER) = Fish weight gain / Protein fed
Percentage survival (%)=Number at the end of experiment / Number at onset of experiment X 100

1.7 Statistical analysis

Biological data resulting from the experiment was subjected to one-way analysis of variance of variance (ANOVA), using the SPSS version 16.0. Duncan's multiple range was used to compare differences among the individual means. Differences were considered at 5% level of significance. A second degree polynomial regression was used to depict the relationship between the SGR (Y) and *L. pentosus* supplementation levels (x) in the experimental fish.

3 RESULTS AND DISCUSSION

3.1 Growth performance and nutrient utilization of African catfish, *Clarias gariepinus* fed with the experiment diets

The growth performance and nutrient utilization of *C. gariepinus* fed with experimental diets with different inclusion level of *L. pentosus* is shown in Table 2. The result showed significant differences ($P < 0.05$) in the mean weight gain, specific growth rate, feed conversion ratio and percentage survival of African catfish, *C. gariepinus* fed with the experimental diet. Fish fed with 10^8 CFU *L. pentosus* showed the best zootechnical performance in terms of highest percentage mean weight gain and specific growth. The best feed conversion ratio was recorded in fish fed 10^8 CFU *Lactobacillus pentosus* which was significantly different from other dietary treatments ($P < 0.05$). Statistically, there was increased growth and nutritional performance of fish in this study with increasing levels of *L. pentosus* ($P < 0.05$) with optimum performance at 10^8 CFU.

Table 2: Zootechnical performance of *C. gariepinus* fed *L. pentosus* supplemented diets

PARAMETERS	T1	T2	T3	T4	T5	T6
IW(g)	30.82 ± 0.89^{c}	30.54 ± 0.16^{c}	31.63 ± 0.35^{bc}	31.18 ± 1.07^{bc}	31.86 ± 0.67^{b}	32.11 ± 0.08^{a}
FW(g)	81.62 ± 2.15^{b}	83.48 ± 1.72^{b}	87.84 ± 1.62^{b}	89.58 ± 2.07^{ab}	92.62 ± 1.53^{a}	88.54 ± 1.27^{a}
MWG(g)	50.80 ± 8.05^{b}	52.94 ± 4.56^{ab}	56.21 ± 5.97^{ab}	58.4 ± 0.99^{ab}	60.72 ± 3.19^{a}	56.43 ± 1.80^{ab}
SGR	2.23 ± 0.60^{ab}	2.39 ± 0.27^{ab}	2.43 ± 0.15^{ab}	2.51 ± 0.10^{ab}	2.54 ± 0.22^{a}	2.41 ± 0.19^{b}
FI (g)	65.71 ± 0.95^{a}	65.40 ± 1.49^{a}	68.96 ± 0.18^{b}	66.34 ± 2.04^{a}	66.61 ± 1.02^{a}	65.53 ± 2.21^{a}
FCR	1.29 ± 0.21^{b}	1.24 ± 0.23^{b}	1.23 ± 0.12^{b}	1.14 ± 0.06^{a}	1.10 ± 0.50^{a}	1.16 ± 0.09^{a}
PER	$1.28.\pm0.20^{b}$	1.35 ± 0.11^{ab}	1.42 ± 1.15^{ab}	1.47 ± 0.20^{ab}	1.53 ± 0.08^{a}	1.43 ± 0.05^{ab}
SURVIVAL (%)	96.33 ± 3.33^{a}	96.00 ± 0.00^{a}	96.67 ± 3.33^{b}	96.67 ± 1.67^{a}	96.00 ± 0.00^{a}	96.00 ± 0.00^{a}

Values on the same row, having different superscripts assigned with Duncan New Multiple Range Test after the ANOVA procedures are significantly different (P < 0.05)

Initial weight= IW, Final weight= Final Weight, Mean Weight Gain = MWG, Specific Growth Rate=SGR, FI=Feed Intake, Feed Conversion Ratio= FCR, Protein Efficiency Ratio = PER

The second degree polynomial regression model depicted that a strong relationship existed between the SGR and *L. pentosus* supplementation levels in the experimental fish. A differential equation ($Y = -0.0047x^2 + 0.0609x + 2.3019$, $R^2 = 0.8326$) (Figure 1) shows that the optimum SGR could be achieved at the approximate level of 10^7 *L. pentosus* supplementation. The lowest mean weight gain from the current study was observed in the control and highest were found in treatment five (T5) with 10^8CFUg^{-1}. However, a differential equation (Figure 1) revealed that the optimum SGR could be achieved at the approximate level of 10^7 *L. pentosus* supplementation This is in agreement with the growth performance and nutrient utilization of African catfish, *Clarias gariepinus* obtained by [5] where *Lactobacillus plantarum* was used as supplements. It was reported that diets with 10^3 and 10^7 CFUg^{-1} *L. plantarum* had better growth performance and nutrient utilization than the control which had no probiotic supplementation. Probiotics have been found to improve growth, protein efficiency ratio, and feed conversion ratio, in rohu, *Labeo rohita* 16]. In the current study, the best FCR values were observed in probiotic-supplemented diets suggesting that, the supplementation of *L. pentosus* improved feed utilization, in practical terms the probiotic supplementation was instrumental in the efficient conversion of feed fed into flesh by the cultured fish. Consequently, there was a decrease in the amount of feed necessary for fish growth which could result in production cost reduction. Similarly, the enhanced protein efficiency ratio in all the fish fed diets with *L. pentosus* suggests that the bacteria may be involved in optimizing the use of dietary protein. This could reduce wastage since more than 70% of the cost of fish feeds is protein [7].

Lara-flores *et al.,* (2003) reported that composite supplementation of *Streptococcus faecium, Lactobacillus acidophilus* and yeast *Saccharomyces cerevisiae* were effective as growth promoters and improve nutrient utilisation in Nile Tilapia (*Oreochromis niloticus*) while [1] reported the same in African catfish fed probiotics supplemented diets. Probiotics are viable cell preparations that have beneficial effects on the nutrition and health of a host by improving its feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, growth promoting factors and an increased immune response [17]. The gastrointestinal microbiota of fish and shellfish are peculiarly dependent on the external environment, due to water flow passing through the digestive tract [10]. Therefore, the beneficial effects of the supplementation of dietary *L. pentosus* can be attributed to the modification of the host associated or ambient microbial community of the gastrointestinal tract thus promoting better feed utilization, enhancing the host's response towards disease and improving the quality of its ambient environment [4]. The physicochemical parameters of water observed in this study were temperature, dissolved oxygen, and PH and the values were within the acceptable ranges recommended for pisciculture [18].

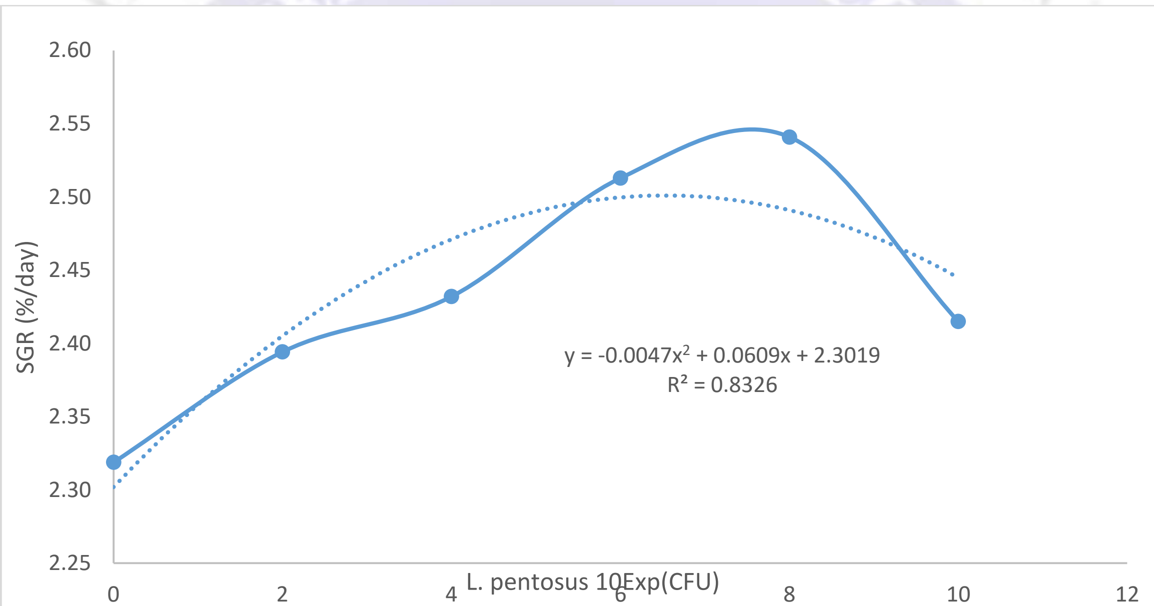

Figure 1: Second degree polynomial regression analysis of SGR of fish fed *L. pentosus* supplemented diets

3.2 Haematological parameters of fish fed *l. pentosus* supplemented diets

The result of the haematological parameters of African catfish, *C. gariepinus* in the experimental trial was presented in Table 3. There was significant difference (P < 0.05) in the haematological parameters of fish fed the experimental diet. The lowest PCV was recorded in the control (T1) and increased with increasing levels of probiotic in the diet of fish. The highest WBC was recorded in T2, the lowest was recorded in T6. The highest RBC was recorded in T5, the lowest RBC was observed in T1. The highest leucocytes were recorded in T6 and the lowest was observed in the control. The haematology result of present study showed that WBC, RBC, PCV and other differential blood parameters were affected by the supplementation of *L. pentosus* in *C. gariepinus* diet. This is in agreement with [19] who stated that there was an increase in RBC, WBC, and PCV in fish groups fed with diet supplemented with probiotic (*B. subtillis* and *Saccharomyces cerevisae*). They concluded that probiotics can be considered as an alternative to antibiotics for feed supplement and health improvement in aquaculture. Haematological characteristics have been widely used in clinical diagnosis of disease and pathologies of human and domestic animals. The applications of haematological techniques have proved valuable for fishery biologists in assessing the health of fish and monitoring stress responses. [20] reported that the haematological parameters of fish are affected by 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 current study showed that *L. pentosus* supplementation improved the blood parameters and health of fish.

Table 3: Haematological parameters of fish fed the experimental diet

Parameters	T1	T2	T3	T4	T5	T6
PCV (%)	21.00±0.58^{a}	22.67±0.88^{ab}	23.67±0.88^{bc}	25.00±0.58^{cd}	26.00±0.58^{d}	25.00±0.58^{cd}
WBC (×10^{2} ml)	80.00±2.88	83.50.±2.59^{d}	73.00±2.88^{c}	71.00±1.15^{c}	62.00±5.77^{b}	53.50.00±1.43^{a}
RBC (×10^{2} ml)	2.35±0.06^{a}	2.53±0.10^{ab}	2.65±0.87^{bc}	2.80±0.06^{cd}	2.90±0.06^{d}	2.80±0.06^{cd}
Neutrophils (%)	66.00±0.58^{e}	66.33±0.88^{a}	63.00±2.89^{bc}	67.33±0.88^{de}	63.00±0.58^{cd}	58.00±1.15^{ab}
Leucocytes (%)	29.67±0.88^{a}	30.00±0.58^{d}	33.00±1.15^{b}	29.00±1.73^{a}	33.00±0.58^{c}	41.67±1.46^{d}
Monophils (%)	2.00±0.33^{a}	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{a}
Eosinophils (%)	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.33^{a}	2.00±0.00^{a}	1.00±0.00^{b}

Values on the same row, having different superscripts assigned with Duncan New Multiple Range Test after the ANOVA procedures are significantly different (P < 0.05)
PCV- PACK CELL VOLUME, RBC- RED BLOOD CELL, WBC- WHITE BLOOD CELL

4. CONCLUSIONS

In the present study results showed improvement in the growth parameters and feed utilization of African catfish fed with feed containing *L. pentosus* at different supplementation levels. The best supplementation level in terms of FCR was found in T5, with 10^{8}cfu.However, the polynomial regression shows that the optimum SGR could be achieved at the approximate level of 10^{7} *L. pentosus* supplementation. Furthermore, the highest inclusion level of *L.* pentosus did not prove to be the best according to the results obtained from experiment. The haematology parameters also showed that *L. pentosus* does not have negative effect on the various haematological parameters analysed. The blood parameters in this study were positively affected and better than the control diet which had no probiotics supplementation. The result of the present study regarding zootechnical and blood parameters of fish confirms *L. pentosus* as a probiotic which can promote nutrient utilisation and health in *C. gariepinus*. From the above deductions *Lactobacillus pentosus* can be used as an important probiotic in aquaculture.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors

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