



Histometry and Growth Performance of African Catfish, *Clarias gariepinus*, (Burchell, 1822) Fed Probiotics Supplemented Diets

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The histometry, organosomatic parameters and growth performance of African catfish, *Clarias gariepinus* fed with commercial probiotics, *Lactobacillus pentosus* supplemented diet was assessed in the present study. Six experimental diets were formulated, at different inclusion levels of *Lactobacillus pentosus* of 0, 10², 10⁴, 10⁶, 10⁸ and 10¹⁰ cfu in T1, T2, T3, T4, T5 and T6 respectively. *C. gariepinus* were stocked into concrete tanks of 1.93 m by 1.93 m by 1.09 m. Each experimental diet was fed to three replicate groups of fish for 42 days. At the end of the feeding, histometrical parameters like mucosal fold length, mucosal fold breadth, mucosal fold area improved with increasing supplementation levels of *L. pentosus*. There was also significantly better $P > 0.05$ organosomatic parameters in all treatments with increasing level of supplementation. The best growth performance in term of specific growth rate (SGR) and feed conversion ratio (FCR) was observed in Treatment 3 as 10.49±0.23 and 1.56±0.22 respectively. The enhanced growth performance and histometry in fish fed diets with *L. pentosus* supplementation resulted in the best biological performance in African catfish at 1.0 g per 100 g of feed representing 10⁴ cfu supplementation level. Therefore the present study shows that *L. pentosus* can be recommended as an important probiotic in aquaculture.

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1. INTRODUCTION

Fish has always been a potential source of animal protein and essential nutrients in the world, it is needed for the maintenance of a healthy body [1]. Fish are excellent sources of protein when compared with other sources of protein due to the amino acid composition and protein digestibility [2]. They also serve as a favourite foodstuff for large number of people across the globe due to its several health benefits [3]. In fish farming, sufficient consumption of feed is essential for increased yield and profitability and there is now need to search for alternative protein sources for fish feeds especially in developing countries like Nigeria [4,5]. Fish is one of the cheapest and direct sources of protein and micro nutrients for millions of people in Africa [6]. Therefore, to solve the high demand for fish, aquaculture production remains 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 [5].

With the increasing intensification and commercialization of aquaculture production, disease is a major problem in the fish farming industry [7]. Although vaccines are being developed and marketed, they cannot be used as a universal disease control measure in aquaculture. During the last decades, antibiotics were used as traditional strategy for fish diseases management but also for the improvement of growth and efficiency of feed conversion. However, the development and spread of antimicrobial resistant pathogens were well documented [8,9,10]. There is a risk associated with the transmission of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of non-pathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens [11].

Probiotics are live microorganisms which have beneficial effects on the hosts by modifying 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 [12]. Probiotics are viable

cell preparations that have beneficial effects on the 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 [13]. They may play a considerable role as immunostimulants and antimicrobial agents [14]. The research on probiotics for aquatic animals is increasing with the demand for organic aquaculture. The gastrointestinal microbiota of fish and shellfish are peculiarly dependent on the external environment, due to water flow passing through the digestive tract [15]. *Lactobacillus pentosus* is a commercially significant bacteria probiotic, originally isolated from the human gastrointestinal tract and designated *Bacillus pentosus* in 1900. Throughout the development of methods to identify and characterize bacteria, *L. pentosus* has undergone multiple taxonomic revisions and it is presently a phylogenetic subgroup in the highly diverse and heterogeneous *Lactobacillus* [16]. Recently attention has focused on the use of probiotics in aquaculture. Probiotics are used as dietary supplements in aquaculture and their role in intestinal microbial balance, growth, nutrition, health status and resistance against infectious agents are already established [15]. The use of probiotics as feed supplement has attracted considerable attention by feed manufacturers as a means of improving livestock performance, most of the studies concerned with the effect of probiotics on cultured aquatic animals have emphasized a reduction in mortality, increased survival [17], improved resistance against disease [18], enhance the ability to adhere and colonize the gut [19], improved the ability to antagonize other organisms [20,21,22,23].

The African catfish, *Clarias gariepinus* is one of the most important freshwater fish species in Africa [24]. African catfish, *Clarias gariepinus* also known as sharp tooth catfish in aquaculture is a highly recommended food fish in Africa [25]. It is a good converter of feed to flesh and is able to withstand harsh environmental conditions. Another characteristic that makes *C. gariepinus* a good experimental fish is its resistance to diseases and fast growth rate [26]. The aim of the present study is to determine the effects of dietary, lyophilized *Lactobacillus pentosus* a commercial probiotic, on the growth performance and histometry of African catfish, *Clarias gariepinus* post juveniles.

2. MATERIALS AND METHODS

2.1 Preparation of Experimental Diet

The feed ingredients were purchased at Animal concept Feedmill, Bye pass, Akure, Ondo State, Nigeria. Six isonitrogenous diets containing 40% crude protein were formulated for post juvenile of African Catfish. A commercial *Lactobacillus* probiotic product entitled Lactopent which contains *L. pentosus* (Lennux company, USA) was purchased and added to the test the ingredients. Lyophilized *Lactobacillus pentosus* was used as probiotics at 0.00, 10²cfu, 10⁴cfu, 10⁶cfu, 10⁸cfu and 10¹⁰cfu (0.00, 0.5 g, 1.0 g, 1.5 g, 2.0 g and 2.5 g) inclusion levels (Table 1) denoted as T1, T2, T3, T4,T5 and T6 respectively. All dietary ingredients were milled into small particle size. The dry particle was thoroughly mixed by adding hot water until a constant dough is gotten. The dough was pelleted using a Hobart pelleting machine (Hobart Model 200, CA, USA) with a 6.0mm 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 [27].

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.

2.2 Experimental Fish and Feeding Trial

Clarias gariepinus juveniles with average weight of 31.20±0.11g were obtained from the Hatchery unit of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, prior to the feeding trial. Fish were graded by size and groups of 10 fish per replicate were stocked into concrete tanks of 1.93 m by 1.93 m by 1.09 m. A commercial diet, Vital feed (42% crude protein) was fed to all fish during a one - week conditioning period. Each experimental diet was fed to three replicate groups of fish for 42 days. All groups were fed their respective diets at the same fixed rate

(initially 4% of body weight per day). This rate was adjusted each week. Fish were fed by 0900-1000 and 1700-1800 GMT, for 7 days each week. Growth was monitored weekly by batch weighing of fish from each tank. 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).

2.3 Determination of Organosomatic Indices

2.3.1 Hepatosomatic index

At the end of the feeding trial, the fish were removed from each treatment for hepatosomatic index according to [28]. One fish from each treatment was separated for liver samples.

Hepatosomatic index was calculated by:

$$\text{HSI}\% = \text{Liver weight} / \text{Somatic weight} \times 100$$

2.3.2 Intestinosomatic index

At the end of the experimental period, the fish were removed for intestinosomatic index. Fish from each treatment were separated for intestine samples

Intestinosomatic index was calculated by:

$$\text{ISI}\% = \text{Intestine} / \text{Somatic weight} \times 100$$

2.4 Evaluation of Growth Performance

Fish performance during the experiment was based on the productivity indices on growth performance and nutrient utilization efficiencies as described by [29].

Mean weight gain: this was calculated as the difference between the final mean weight and the initial mean weight of the experimental fish.

$$\text{Mean weight gain (g)} = \text{Final mean weight} - \text{Initial mean weight}$$

Specific growth rate: was calculated from the relationship between the differences in the Log weight of fish within the experimental period as follows;

$$\text{SGR (\%)} = (\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight}) / \text{Feeding days} \times 100$$

$$\% \text{ Weight gain} = \text{Final weight} - \text{Initial weight} / \text{Initial weight} \times 100$$

Table 1. Composition and proximate composition of the experimental diet in g/100 g dry matter containing various inclusion levels of *Lactobacillus pentosus*, 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
Soybean 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
<i>Lactobacillus pentosus</i> (cfu)	0.00	10 ²	10 ⁴	10 ⁶	10 ⁸	10 ¹⁰
Parameters	T1	T2	T3	T4	T5	T6
Moisture	10.33±0.75 ^c	10.16±0.00 ^b	10.53±0.20 ^d	10.12±0.01 ^b	9.68±0.00 ^a	10.45±0.07 ^{ab}
Ash	10.81±0.05 ^b	11.01±0.06 ^b	10.71±0.00 ^b	10.08±0.47 ^a	10.08±0.01 ^b	11.11±0.00 ^b
Lipid	20.73±0.20 ^{ab}	20.00±0.20 ^a	21.00±0.36 ^b	20.85±0.46 ^{ab}	20.40±0.23 ^{ab}	19.98±0.00 ^a
Protein	39.77±0.66 ^a	39.71±0.03 ^a	39.45±0.19 ^a	39.65±0.10 ^a	39.60±0.03 ^a	39.50±0.04 ^a
Fibre	4.43±0.04 ^{bc}	4.32±0.00 ^b	4.35±0.03 ^{bc}	4.16±0.10 ^a	4.35±0.02 ^{bc}	4.50±0.02 ^c
NFE	13.95±0.03 ^a	15.3±0.03 ^d	13.97±0.12 ^a	15.15±0.17 ^{cd}	14.85±0.18 ^b	14.47±0.12 ^b

Mean values on the same row, having different superscripts are significantly different ($P < 0.05$)

Feed Conversion Ratio (FCR)

FCR = Total feed consumed by fish / Weight gain by fish

Percentage survival (%) = Number at the end of experiment / Number at onset of experiment X 100

2.5 Histological Examination and Histometry of Organs

At the end of the experiment, fish from each treatment were used for histological and histometrical analysis. The distal intestine and liver were removed after dissecting the fish and examined. They were fixed in 10% Formalin for three days to preserve the organs. The fixed organs were dehydrated in graded levels of alcohol (50%, 70%, 90%, 100%) after which they were immersed in 50/50 mixture of Alcohol and xylene for three hours, followed by cleaning in 100% xylene for three hours after which they were embedded in petri dishes with wax. The specimens were later mounted on wooden blocks and sectioned with the aid of a microtome to 7µm sections before staining in haematoxylin and eosin. The stained specimens were observed under a light microscope, this was carried in accordance to the method used by [26]. Histometrical measurement was performed according to [30], using the following parameters, mucosal fold, length, mucosal fold breadth and mucosal fold area in micrometers and millimeters.

2.6 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 (Statistical package computer software) Duncan's multiple range was used to compare differences among the individual means [31]. Differences were considered significant at P-levels (< 0.05).

3. RESULTS

The proximate composition of the experimental diets (Table 1) showed that the protein content of the diets was not significantly different ($P > 0.05$) confirming that the experimental diet is isonitrogenous. The ash content of the diets was not significantly different at $P > 0.05$ for T1, T2, T3, T5 and T6, except for T4 it was significantly different at $P > 0.05$. The lipid content of the feed was not significantly different at $P < 0.05$ for T1,

T4, and T5, and between T2 and T6. The growth performance and nutrient utilization of *C. gariepinus* fed with experimental diets that contain different inclusion level of *L. pentosus* is presented in Table 2. The result showed differences in the mean weight gain, specific growth rate, feed conversion ratio and percentage survival of African catfish, *C. gariepinus* post juveniles fed with the experimental diet. Fish fed with 10^4 CFU *L. pentosus* showed the highest mean weight gain and specific growth rate, 28.26 ± 0.89 and 10.49 ± 0.23 respectively. However the best feed conversion ratio was recorded in fish fed 10^4 CFU *Lactobacillus pentosus* which was significantly better than 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* ($P < 0.05$) up to the inclusion level of 10^4 CFU *Lactobacillus pentosus*. Feed related mortality was not observed during the feeding trials.

The result of the histometry and organosomatic indices at the end of the experimental period was shown in Table 3. There was significant difference ($P < 0.05$) in the mucosal fold length across all treatments. However, there was no significant difference ($P > 0.05$) for mucosal fold breadth between T1 and T3, T2 and T5. There was significant difference ($P < 0.05$) in the organosomatic indices of all treatments with increasing level of supplementation. There was no significant difference $P > 0.05$ for intestinosomatic index between T1, T3 and T4. There was significant difference $P > 0.05$ for hepatosomatic index of fish in all treatments (Table 3). The histology of fish fed probiotics supplemented diets in Fig. 1 (Plate a-f) showed apparently better enterocytes and more intestinal villi in fish fed the probiotics supplemented diets than the control.

4. DISCUSSION

Result from the current study showed that the best growth performance was observed in fish fed with 10^4 cfug⁻¹ in treatment four. The lowest growth performance was recorded in fish fed the control diet, this is in agreement with [32] who reported that *Lactobacillus* and *Bifidobacterium* used as probiotics improved the growth performance of *C. gariepinus* over the control diets. The best FCR values were observed in probiotic-supplemented diets suggesting that the addition of probiotics improved feed utilization, in practical terms this means that probiotic used can decrease the amount of feed necessary for

Table 2. Growth performance of *Clarias gariepinus* fed probiotics supplemented diets

Parameter	T1	T2	T3	T4	T5	T6
IW	12.06±0.34 ^a	12.11±0.22 ^a	11.85±0.09 ^a	11.91±0.41 ^a	11.92±0.30 ^b	12.08±0.38 ^a
FW	37.32±0.32 ^a	39.27±0.67 ^{ab}	40.11±0.72 ^b	39.34±13.48 ^{ab}	38.31±0.63 ^{ab}	38.87±0.74 ^{ab}
MWG	25.26±0.19 ^a	27.16±0.46 ^{ab}	28.26±0.81 ^b	27.43±0.94 ^b	26.39±0.89 ^{ab}	26.79±0.10 ^{ab}
SGR	9.63±0.01 ^a	10.16±0.15 ^{ab}	10.49±0.23 ^b	10.45±0.28 ^b	10.24±0.29 ^{ab}	9.77±0.27 ^b
FCR	1.72±0.01 ^c	1.58±0.17 ^c	1.56±0.22 ^{ab}	1.57±0.19 ^{ab}	1.64±0.28 ^{ab}	1.72±0.22 ^a
PI	63.21±0.67 ^{bc}	64.21±0.43 ^c	63.71±0.18 ^c	62.37±0.81 ^{bc}	61.65±0.53 ^b	59.07±0.61 ^a
PER	0.90±0.01 ^a	1.11±0.06 ^a	1.30±0.12 ^{ab}	1.30±0.13 ^{ab}	1.22±0.15 ^{ab}	1.58±0.19 ^b
PS	90.00± 0.00 ^b	93.67±8.82 ^b	97.33±3.33 ^b	96.43±2.33 ^a	95.35±3.33 ^b	93.33± 6.67 ^b
FI	14.64±0.95 ^e	17.24±0.49 ^{de}	17.49±0.18 ^d	17.47±2.04 ^a	16.06±1.02 ^c	15.55±0.21 ^b

Mean Values On The Same Row, Having Different Superscripts Are Significantly Different ($P < 0.05$); *Iw*: Initial Weight, *Fw*: Final Weight, *Mwg* : Mean Weight Gain, %*Mwg*: % Mean Weight Gain, *Fi* : Feed Intake, *Sgr* : Specific Growth Rate, *Fcr*: Feed Conversion Ratio, *P.S*: Percentage Survival, *Per*: Protein Efficiency Ratio

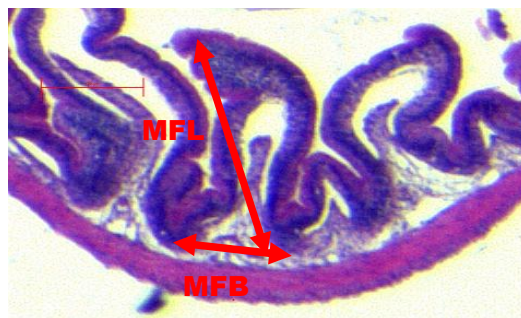
Table 3. Histometry and organosomatic indices of experimental fish fed probiotics supplemented diets

Parameter	T1	T2	T3	T4	T5	T6
MFL (μm)	401.67±4.42 ⁱ	376.33±0.88 ^d	388.00±2.89 ^e	352.00±2.30 ^b	361.33±2.33 ^c	323.33±1.45 ^a
MFB (μm)	117.67±1.76 ^a	139.67±0.88 ^b	118.33±1.45 ^a	158.00±1.15 ^c	138.67±1.45 ^b	212.67±0.67 ^d
MFA (mm)	47.33±0.33 ^a	52.67±0.33 ^c	46.00±0.00 ^a	55.67±0.88 ^d	49.67±0.33 ^b	68.67±0.33 ^e
Intestinosomatic	0.40±0.02 ^a	0.44±0.02 ^{bc}	0.39±0.01 ^a	0.48±0.02 ^c	0.42±0.00 ^a	0.45±0.01 ^{bc}
Hepatosomatic	0.48±0.02 ^a	0.51±0.01 ^a	0.49±0.01 ^a	0.50±0.01 ^a	0.65±0.01 ^b	0.63±0.01 ^b

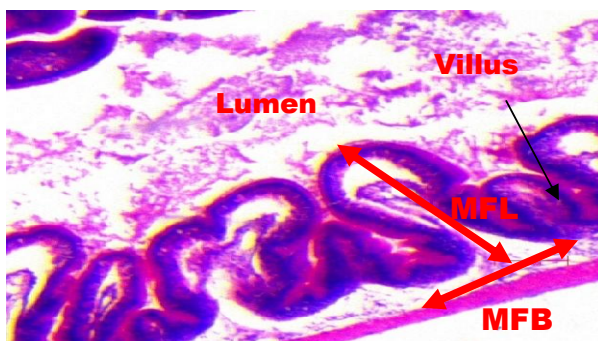
Mean values on the same row, having different superscripts are significantly different ($p < 0.05$); *mfb*: mucosal fold length *mfb*: mucosal fold breadth, *mfa*: mucosal fold area



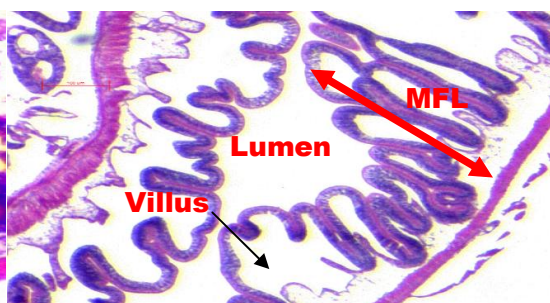
a: Photomicrographs of the cross section of the posterior intestine of African catfish in the control (Treatment 1) showing lumen, villus, mucosal fold length (MFL), mucosal fold breadth (MFB.)



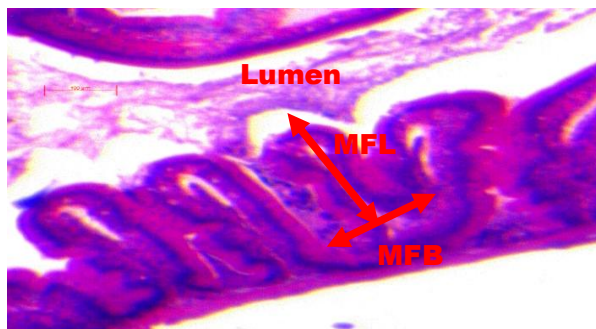
b: Photomicrographs of the cross section of the posterior intestine of African catfish (Treatment 2) showing normal lumen, villus, mucosal fold length (MFL), mucosal fold breadth (MFB)



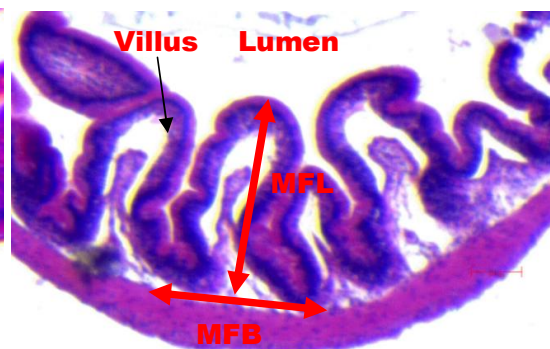
c: Photomicrographs of the cross section of the posterior intestine of African catfish (Treatment 3) showing normal lumen, villus, mucosal fold length (MFL), mucosal fold breadth (MFB)



d: Photomicrographs showing the cross section of the posterior intestine of African catfish (Treatment 4) showing normal lumen, villus, mucosal fold length (MFL), mucosal fold breadth (MFB)



e : Photomicrographs of the cross section of the posterior intestine of African catfish (Treatment 5) showing normal lumen, villus, mucosal fold length (MFL), mucosal fold breadth (MFB)



f : Photomicrographs of the cross section of the posterior intestine of African catfish (Treatment 6) showing normal lumen, villus, mucosal fold length (MFL), mucosal fold breadth

Fig. 1. Photomicrographs of the cross section of the posterior intestine of African catfish, *Clarias gariepinus* (Burchell, 1822) fed probiotics supplemented diets the histology of fish fed probiotics supplemented diets showed apparently better enterocytes and more intestinal villi than the control

animal growth which could result in production cost reduction. Similar results have been reported by [33] who used *Streptococcus faecium*, *Lactobacillus acidophilus* and yeast *Saccharomyces cerevisiae* as growth promoters in Nile Tilapia (*Oreochromis niloticus*). The enhanced growth performance 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.

Geovanny et al. [34] described the feeding of probiotics as a way of improving the appetite and growth performance of the farmed fish. The improvement in fish growth and feed utilization observed in this study showed that probiotic supplemented diets may be linked to improved nutrient digestibility and this could be as a result of the ability of certain enzymes capable of converting certain component of the diet into a more digestible nutrient for the host. The growth performance and nutrient utilization of African catfish, *Clarias gariepinus* obtained in the current work also agree with [35] who reported that *Lactobacillus plantarum* when used in supplementation of fish diets improved growth performance and nutrient utilization. This is also in line with other authors like [36]. [37] who reported the use of *Bacillus coagulans*, *Rhodospseudomonas palustris* and *Lactobacillus acidophilus* as growth promoters in grass carp (*Ctenopharyngodon idella*) fingerlings. Similarly [23] reported that *L. Plantarium* improved the nutritional performance in *C. gariepinus* with increasing levels upto 10^5 CFU.

The good growth performance and nutrient utilization of the fish fed diets supplemented with *Lactobacillus* species over those fed diets without the bacteria has been confirmed using different types of probiotics individually or symbiotically [37]. The effect of *L. pentosus* may be linked to improvement in the intestinal microbial balance of fish [38] that out-compete the pathogenic bacteria by boosting the gastrointestinal tract and hence reducing the pathogenic flora thus leading to increased food absorption [39]. The inclusion of probiotics in diets may significantly help to stimulate appetite and improve nutrition through production of vitamins, detoxifying toxic compounds in the diets, and also aid the breaking down of indigestible compounds [39]. Moreover, probiotics increase the protease, amylase, and cellulose activities in grass carp (*Ctenopharyngodon idella*) and hence improve diet digestibility, which might in turn explain

improved growth in fish fed diets supplemented with probiotics [36].

This present study showed that the inclusion of *L. pentosus* in the experimental diet did not only affect the histometry of the intestines of *C. gariepinus*, but also modified it by improving a higher mucosal area, which provided a larger surface area for absorption and utilization of nutrients. This is supported by the report of [30] which stated that the enterocyte structure are responsible for the nutritional performance of rainbow trout (*Oncorhynchus mykiss*). The intestinal mucosa in African catfish, *Clarias gariepinus* was characterized by the presence of longitudinal folds; in the anterior part of the intestine of catfish, there were numerous mucosal folds that may allow maximal distension for the prey and broken down food, and it was lined by stratified epithelium with mucous secreting cells [26]. Therefore, the activity of the enterocytes were more pronounced in fish fed the *L. pentosus* supplemented diets. This corroborated the study of [40] on African catfish which concluded that the higher number of nucleus in the enterocytes signified the presence of more active digestive cells which improved the digestion and nutrient utilization in African catfish.

5. CONCLUSION

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 inclusion levels. The best supplementation level is T3, 10^4 CFU in terms of FCR, because it had a lower feed conversion ratio (FCR). The highest inclusion level of *L. pentosus* did not prove to be the best according to the results obtained from experiment. The histometrical studies in this work also revealed that the intestine was positively affected and performed better than the control diet which had no probiotics supplementation. From the above deductions *Lactobacillus pentosus* can be used as an important probiotic in aquaculture.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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