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Influence of *Lactobacillus plantarum* supplemented diet on growth response, gut morphometry and microbial profile in gut of *Clarias gariepinus* fingerlings

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ABSTRACT

Objective: To evaluate the influence of dietary inclusions of *Lactobacillus plantarum* (*L. plantarum*) on growth response, gut morphometry and intestinal microbial profile of *Clarias gariepinus* (*C. gariepinus*) fingerlings was carried out using a total of 150 *C. gariepinus* fingerlings (2.35 ± 0.48 g/fish) by selecting at random into five treatments groups of 10 fish in 3 three replicates each.

Methods: *L. plantarum* isolated from corn slurry was cultured using standard measures. Five isonitrogenous diets were prepared at 35% crude protein (T_0, T_1, T_2, T_3 and T_4) with *L. plantarum* at inclusion rate of 0.0%, 0.5%, 1.0%, 1.5% and 2.0% respectively. The fish were fed at 5% body weight per day for 12 weeks twice daily.

Results: T_4 recorded the highest mean weight gain and specific growth rate while the lowest was obtained in T_1. T_4 (1.97) when compared with other treatments had marginally lower feed conversion ratio. Absorptive area was most significantly higher in T_3 and T_4 group when compared to the control (T_0) and other lower dietary probiotic inclusion groups. Cryptal depth was highest in T_4 with significant difference which also gave the maximum enterobacteriaceae count while T_0 recorded the least count.

Conclusions: From these indications, *L. plantarum* fortified diets may enhance the growth of cultured *C. gariepinus* fingerlings at 2.0% inclusion rate as it was observed to improve body weight gain, feed conversion ratio with increment in the absorptive area and the microbial count in the gut.

1. Introduction

Of all animal food sectors, aquaculture has been reported to be considered to have more rapid growth than all others[1]. With such increase in growth of aquaculture productions comes challenges of developing suitable feeds and enhance management of water quality[2]. Enhancing the digestibility of feedstuffs by addition of probiotic is an initiative that has been proven to increase the availability of absorbable nutrients to animals[3]. This is especially true in fish where maximizing the absorbable nutrients in commercial feeds has being the forefront of feed composition and production. Hence the use of probiotics in improving appetite stimulation and digestibility, have been a focus of research especially as some have been reported to help produce vitamins and aid degradation of indigestible compounds[4]. In Nigeria, catfish is widely cultured[5] because of its high growth rate, hardiness to disease and environmental conditions and ability to easily spawn. African catfish is one of the most suitable species and appreciated in a wide number of African consumers[6].

Probiotics are live microbes which when administered in right dosage, become attached to the digestive tract forming a thin biofilm. The beneficial outcome on the host includes improved digestibility of feed materials, protection and resistance to diseases, as they produce substances such as lactic acid and bacteriocins, which inhibits the growth of harmful bacteria[7,8]. As desire for environment friendly aquaculture rises, there has been an increase in the use of probiotics in aquaculture[9,10] as their role in stimulating nutrient digestibility, enhance weight gain in fish and shrimp cultures has been established[11].

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All experimental procedures which involves animals were carried out in accordance with the Nigerian constitution and the National Health Research Ethics Code (NRHEC) and approved by Animal Care and Use Research Ethics Committee (ACUREC).

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Lactobacilli are non-pathogen to facultative organisms which have been widely explored as probiotics in aquaculture[12]. Lactic acid bacteria (LAB) have been found to be great producers of bacteriocins and organic acids which inhibit *in vitro* the growth of some harmful microbes in fish[13]. The presence of these antimicrobial substances has provided LAB with a better advantage over other microorganisms to be used as probiotics[14].

Lactobacillus plantarum (*L. plantarum*) is one of the bacteria isolated and connected with the fermentation of corn for the production of fermented corn slurry, locally called "Ogi" in Southwestern Nigeria[15]. It has been recognized as the most popular conventional health-sustaining fermented food in Western Nigeria[16] which is mainly prepared from cereals which include but not limited to white/yellow maize (*Zea mays*), white guinea corn (*Sorghum bicolor*), and millet (*Pennisetum typhoideum*)[17]. The nutritional benefits of corn slurry have been evaluated[15]. However, there has been dearth of information on the use of LAB isolated from fermented food in African catfish for growth response and nutrient digestibility. This present study was therefore aimed to examine the influence of *L. plantarum* isolated from fermented corn slurry on growth response, gut morphometry and profile of microbial flora in the gut *Clarias gariepinus* (*C. gariepinus*) fingerlings.

2. Materials and methods

2.1. Bacteria culture propagation

Pure culture of *L. plantarum* was obtained from the Department of Microbiology, University of Ibadan which was previously isolated from fermented corn slurry (Ogi). Then 10^9 CFU/mL of *Lactobacillus* were inoculated into 10 mL de Man Rogosa Sharpe broth medium (Oxoid, Hampshire, UK), and incubated at 37 ˚C for 24 h. Ten milliliter cultured broth was added into 90 mL of sterile MRS broth (Oxoid, Hampshire, UK) and incubated at 37 ˚C for 24 h and subsequently scaled up to obtain 250 mL broth culture. Cells were then extracted by centrifugation at 4000 r/min for 15 min, supernatant was discarded and rinsed twice with sterile distilled water before suspension in 250 mL to obtain 10^9 CFU/mL culture meant for inoculation of each of the fish tank. The cell suspensions were inoculated into fish feed at 0.5%, 1.0%, 1.5% and 2.0% inclusion respectively in feed.

2.2. Preparation of experimental diets and water system

Five experimental diets were fortified with *L. plantarum* at different inclusion doses. The diet included fish meal, soybean meal and groundnut cake as the protein source and yellow corn as the carbohydrate source (Table 1). The milled dry componenents and the cultured bacteria were mixed with water for 10 min. Then 5 mL, 10 mL and 15 mL and 20 mL of LAB at 10^7 CFU/mL per 100 g feed were used for dietary treatment groups T_1, T_2, T_3, and T_4 respectively while the control T_0, was without probiotics. The dietary feed was pelletized, dried at room temperature for 48 h, and stored in a cool dry place. The proximate analysis of the experimental diet revealed 36% crude protein, 16% ether extract, 15% nitrogen free extract, 17.0% ash, and 14.7% moisture. All the dietary feed meets the nutrient demand for growth of *C. gariepinus* fingerlings as recommended by Adewolu *et al*.[6].

Table 1
Compositions of experimental diet (100 g) feed.

Ingredients	T_0	T_1	T_2	T_3	T_4
Fish meal (65%)	20.00	20.00	20.00	20.00	20.00
Soybean meal	20.50	20.50	20.50	20.50	20.50
Groundnut cake	27.00	27.00	27.00	27.00	27.00
Starch	5.00	5.00	5.00	5.00	5.00
Yellow corn	20.00	20.00	20.00	20.00	20.00
Fish premix	2.00	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Oyster shell	5.00	5.00	5.00	5.00	5.00
Probiotics	-	0.50	1.00	1.50	2.00

T_0: Control; T: Treatment.

The experiment was conducted in fifteen tanks (26 cm × 46 cm × 20 cm) for twelve weeks. Each plastic tank was filled with dechlorinated tap water. In each tank, the water level was maintained at 25 L and was periodically cleaned and replaced every day.

2.3. Experimental procedure and feeding trials

A total of 150 healthy *C. gariepinus* fingerlings having an average weight of (2.35 ± 0.48 g/fish) were procured from a reputable fish farm at Ibadan. The Fish were acclimatized for two weeks prior to the experiment and fish were fed twice daily at 5% of the body weight[18,19] with commercial feed (35% protein and 7% lipid). Fish were randomly allocated to five treatments (T_0, T_1, T_2, T_3, and T_4) at the end of the adaptation period. Each treatment contained ten fingerlings per tank with three replicates. The fish in each plastic tank were fed with experimental diet. The weight changes were measured biweekly while feeding was modified to the body weight. The average from three replicates of the same treatment was used to compute growth performance parameters. Water quality parameters (dissolved oxygen 4.5–5.5, pH 6.10–9.17, and temperature 26–29 ˚C) were also monitored and ensured to be within tolerance level[20].

2.4. Growth Indices

Fish were evaluated fortnightly during the 12 weeks of experimental period. Total weight was determined to the nearest gram. The growth performance parameters was calculated[21].

Mean weight gain = Average final body weight - Average initial body weight

Feed conversion ratio = Dry weight of feed intake (g)/weight gain

of fish (g)

Feed intake = Total feed intake/number of fish stocked

Specific growth rate = 100 (Log_e final body weight - log_e initial body weight)/time (days)

Protein intake = Feed intake × % of protein in diet

Protein efficiency ratio = Mean weight gain (g)/protein consumed

Percentage weight gain = Mean weight gain (g)/mean initial weight × 100

2.5. Proximate analysis

The proximate composition of fish carcass and experimental diets were examined as recommended by Association of Official Analytical Chemists[22].

2.6. Histomorphometry

The intestinal sections of *C. gariepinus* were prepared according to standard procedure in the Histopathology Laboratory of Department of Veterinary Pathology, University of Ibadan, Nigeria. Photomicrographs and the gut morphometry were measured with the aid of light microscope and Acuscope (TS view)[23,24]. Five different villi were measured and average values were calculated per parameter.

2.7. Enterobacteriaceae count

Intestinal sections of *C. gariepinus* of each treatment were taken at the end of the feed trial to Microbiology Laboratory of Department of Microbiology, University of Ibadan. Bacterial isolates were procured from the gut of the fish using pour plating method[25]. Bacteria colonies which developed after incubation was counted on each of the media used using pour plate method and were expressed in CFU/g.

2.8. Statistical analysis

Growth response, enterobacteriaceae count and gut morphometric values were subjected to One-way ANOVA using SPSS version 17.0. Duncan multiple range test was also employed to assess the differences among individual means.

3. Results

Table 2 presents the proximate composition of experimental diet. The percentage crude protein ranged from 36.21% to 36.40%. The highest crude fibre was found in T_0 and the lowest in T_2 while the percentage fat ranged from 5.02% to 5.05%.

Table 2
Proximate composition of experimental diet.

Treatments	T_0	T_1	T_2	T_3	T_4
Moisture (%)	14.680	14.630	14.600	14.640	14.620
Crude protein (%)	36.400	36.270	36.210	36.300	36.250
Fat (%)	5.050	5.030	5.020	5.040	5.030
Ash (%)	17.000	16.940	16.910	16.950	16.930
Crude fibre (%)	12.810	12.770	12.740	12.780	12.760
% Nitrogen free extract	14.060	14.360	14.520	14.290	14.410
Gross energy (kcal/g)	309.461	309.773	309.998	309.749	309.866

T_0: Control; T: Treatment.

Table 3 shows the growth response of *C. gariepinus* fingerlings fed diets supplemented with different levels of probiotics, *L. plantarum* from corn slurry for 12 weeks. Although general increase in weight gain was observed from T_4 (139.00 ± 15.52) to T_3 (124.33 ± 36.55) to T_0 (123.00 ± 9.53) to T_2 (122.00 ± 19.46) and T_1 (114.33 ± 17.47) but these were not significantly different ($P > 0.05$) from the control (T_0). The highest feed conversion ratio was recorded in T_4 diet group (1.97 ± 0.08) while the lowest was observed in T_3 diet group (2.25 ± 0.48), but in all, feed conversion ratios between the treated groups were not significantly different ($P > 0.05$). The specific growth rate ranged from (2.19 ± 0.11) in T_4 to (1.94 ± 0.10) in T_1 with no significant difference. Protein efficiency ratio ranged from (1.40 ± 0.06) in T_4 to (1.26 ± 0.26) in T_3.

Table 4 presents the proximate composition of the whole fish carcass at the end of twelve weeks of experiment. The highest crude protein was recorded for fish carcass fed with diet T_0 and T_4 while the lowest crude protein was obtained in carcass of fish fed T_2. The

Table 3
Growth performance of *C. gariepinus* fingerlings fed diets with varying levels of probiotics inclusion.

Parameter	T_0	T_1	T_2	T_3	T_4
Initial weight (g)	24.33 ± 0.57[a]	24.00 ± 1.00[a]	23.66 ± 0.57[a]	23.00 ± 1.00[a]	22.33 ± 0.57[a]
Final weight (g)	147.33 ± 10.01[a]	138.33 ± 18.44[a]	145.66 ± 19.08[a]	147.33 ± 37.54[a]	161.33 ± 15.50[a]
Weight gain (g)	123.00 ± 9.53[a]	114.33 ± 17.47[a]	122.00 ± 19.46[a]	124.33 ± 36.55[a]	139.00 ± 15.52[a]
Percentage weight gain (%)	505.16 ± 30.29[a]	474.93 ± 54.57[a]	516.63 ± 91.30[a]	536.72 ± 134.76[a]	622.70 ± 73.14[a]
Specific growth rate (%)	2.00 ± 0.05[a]	1.94 ± 0.10[a]	2.01 ± 0.17[a]	1.98 ± 0.32[a]	2.19 ± 0.11[a]
Feed conversion ratio	2.09 ± 0.14[a]	2.17 ± 0.22[a]	2.04 ± 0.29[a]	2.25 ± 0.48[a]	1.97 ± 0.08[a]
Daily feed intake (g)	1.58 ± 0.05[a]	1.69 ± 0.11[a]	1.62 ± 0.14[a]	1.79 ± 0.26[a]	1.65 ± 0.03[a]
Daily weight gain (g)	0.14 ± 0.01[a]	0.14 ± 0.01[a]	0.13 ± 0.02[a]	0.14 ± 0.03[a]	0.15 ± 0.01[a]
Protein intake (g)	88.74 ± 2.65[a]	89.36 ± 5.05[a]	87.76 ± 3.09[a]	97.63 ± 8.72[a]	99.20 ± 7.22[a]
Protein efficiency ratio	1.39 ± 0.07[a]	1.27 ± 0.13[a]	1.39 ± 0.22[a]	1.26 ± 0.26[a]	1.40 ± 0.06[a]
Feed intake (g)	243.80 ± 7.30[a]	246.36 ± 13.91[a]	245.50 ± 7.56[a]	269.70 ± 24.09[a]	273.66 ± 19.91[a]
Survival rate (%)	100.00	90.00	100.00	96.66	100.00

Mean in the same row with the same superscript are not significantly different from each other. T_0: Control; T: Treatment.

highest content of lipid was obtained in carcass of fish fed diet T_0 (5.82) while the lowest lipid content was obtained in carcass of fish fed diet T_2, T_3, T_4 and T_1.

Table 4
Chemical composition of experimental fish after 90 days of feeding trial.

Parameter	Initial	T_0	T_1	T_2	T_3	T_4
Crude protein (%)	61.73	65.67	65.43	65.33	65.49	65.40
Lipid (%)	9.90	5.82	5.80	5.79	5.81	5.80
Ash (%)	16.40	16.33	16.31	16.31	16.35	16.33
Moisture (%)	12.16	12.51	12.47	12.45	12.48	12.46

T_0: Control; T: Treatment.

Modifications in villi length, width and cryptal depth of *C. gariepinus* fingerlings given diets supplemented with *L. plantarum* from corn slurry for twelve weeks could be seen in Table 5. The result showed that the highest villi length was in T_4 (2 560.95 ± 519.47), while the lowest villi length was observed in T_3 and T_2 (1 667.15 ± 380.65). Treatments T_3 (1 397.13 ± 321.13), T_2 (1 127.26 ± 406.79), T_4 (975.89 ± 185.40) and T_1 (915.30 ± 133.12) showed increased villi depth while the lowest was observed in T_0 (666.22 ± 464.28). The highest absorption area was observed in T_3 (2.79 ± 0.66) and T_4 (2.52 ± 0.85) while the T_0 (1.60 ± 1.26) recorded the lowest area of absorption. Cryptal depth was not significantly different ($P > 0.05$) in T_0, T_1, T_2, and T_3 but there was a significant difference between T_4 (2.0 g probiotic) and T_0 (control) when compared.

Table 5
Changes in villi length, villi width and cryptal depth of *C. gariepinus* fingerlings after 90 days of feeding trial.

T	Villi length (μm)	Villi width (μm)	AA (μm^2)	Cryptal depth (μm)
T_0	2 090.26 ± 660.65^b	666.22 ± 464.28^c	1.61 ± 1.26^c	486.31 ± 242.39^b
T_1	2 152.01 ± 378.74^b	915.30 ± 133.12^b	1.97 ± 0.43^{bc}	462.56 ± 246.96^b
T_2	1 667.15 ± 380.65^c	1 127.26 ± 406.79^b	1.91 ± 0.81^c	539.35 ± 208.55^b
T_3	1 998.25 ± 154.45^b	1 397.13 ± 321.13^a	2.79 ± 0.67^a	465.94 ± 250.46^b
T_4	2 560.95 ± 519.47^a	975.89 ± 185.40^b	2.53 ± 0.85^{ab}	745.21 ± 376.07^a

Mean in the same row with the same superscript is not significantly different from each other. AA: Absorptive area (villi length × villi width) (μm^2) × 10^6. T_0: Control; T: Treatment.

Table 6 however, presents the results of total bacterial count and enterobacteriaceae count of *C. gariepinus* fingerlings after 90 days of feeding trial. The results of total bacterial count isolated from *C. gariepinus* digestive tract pre and post administration of diets fortified with probiotics indicated that the total bacteria and enterobacteriaceae counts increased across the treatment groups when compared to the initial bacteria count. The highest total bacteria count was found in T_4 (94.66 ± 12.22), while the lowest was found in T_0 (50.66 ± 16.25) so also was the enterobacteriaceae count, (T_4 = 51.33 ± 9.71), (T_0 = 38.00 ± 12.52). However, It was observed that enterobacteriaceae count in T_4 (2.0 g probiotic) was significantly different ($P < 0.05$) from T_0, T_1, T_2 and T_3.

4. Discussion

From this study the influence of *L. plantarum* with regards to growth performance, gut morphometry and intestinal microbial profile of *C. gariepinus* fingerlings was elucidated. Result indicated that rate of growth in *C. gariepinus* was enhanced with increasing dose of probiotics resulting in significant increase ($P < 0.05$) in villi length and width as well as the absorptive surface area of the intestine. The highest cryptal depth was developed in T_4 which was significantly higher, while the least value was recorded in T_1. T_4 gave the highest enterobacteriaceae count while T_0 recorded the least count. This in turn signifies an increased turnover rate of enterocytes lining the villi in the group with highest cryptal depth (T_4) which also had the highest supplemented concentration of probiotics.

The efficiency of probiotics in aquaculture is dependent on factors such as aquatic organisms, temperature of the organism, and water quality[26]. The water quality parameters (dissolved oxygen 4.5–5.5, pH 6.10–9.17 and temperature 26–29 °C) were in agreement with Nimrat *et al.*[27] who observed that the optimum growth of African catfish requires temperature, 27–30 °C, at least 5 mg/L of dissolved oxygen and 6.5–9.0 pH in the rearing water. During the period of experiment, diet stored before use showed that moulds quickly developed in control diet and there was no mould found in the diet containing probiotics. This was in agreement reports from Association of Official Analytical Chemists[22] who stated that probiotics contribute to the destruction of moulds, viruses and parasites in feed.

All experimental fish had an increase in weight gain confirming the nutrient adequacy of the experimental diet. Higher growth rate obtained for the test diets could be due to the fact that probiotics are sometimes considered to have a direct growth enhancing outcome on fish. This could either be indirectly by involving in nutrient absorption or directly by supplying nutrients to the fish[28]. Although, no significant difference was found between the treatment groups, this is in tandem with Aderolu *et al.*[29] reports where he stated that the inclusion of *Lactobacillus* in the diet of *C. gariepinus* juveniles resulted in better growth compared with the control. Also, LAB positively affects the growth and specific growth rate in *Nile tilapia* [*Oreochromis niloticus* (*O. niloticus*)][30]. According to this report, growth performance, specific growth rate and feed conversion ratio were significantly higher in fish maintained on probiotic supplemented diets compared with those on the control diet.

Most probiotics inhabit the host gut, influence the digestive

Table 6
Enterobacteriaceae count of experimental fish after 90 days of feeding trial.

Treatments	Initial	T_0	T_1	T_2	T_3	T_4
Total bacteria count (10^3 CFU/g)	39.00 ± 11.10^{bc}	50.66 ± 16.25^{bc}	71.66 ± 21.36^{ab}	74.33 ± 22.74^{ab}	86.17 ± 26.40^{ab}	94.66 ± 12.22^a
Enterobacteriaceae count (10^3 CFU/g)	22.00 ± 5.56^c	38.00 ± 12.52^{ab}	45.00 ± 12.28^{ab}	45.00 ± 23.06^{ab}	48.00 ± 9.50^{ab}	51.33 ± 9.71^a

Mean in the same row with the same superscript is not significantly different from each other. T_0: Control; T: Treatment.

processes by augmenting population growth and production of microbial enzymes, consequently, improving microbial balance of the gut in their favor. This therefore aids nutrient absorption and utilization of feed[31]. The inhabiting rate of bacteria in the gastro-intestinal tracts has been reported depending on the amount of bacteria in the feed[32]. Results from our study validates this report as enterobacteriaceae count in the digestive tract of experimental fish increased in the treated groups (T_1, T_2, T_3 and T_4) compared with the control (T_0) with the highest significant increase found in the highest supplemented group (T_4) and lowest in the control. Increase in microbial flora may have stimulated higher mean weight gain, specific growth rate, utilization efficiencies, and could possibly increase survival and immunity of the fish especially if challenged with pathogenic bacteria. Although the latter claim remains to be validated experimentally with this probiotic species but experiments with other probiotic species have been proven to be protective against diseases[33]. Increase in enterobacteriaceae count of the gut of fish may also explain the improvements observed in the absorptive area and feed utilization in *C. gariepinus* fingerlings. This may be explained by Sayed *et al.*[34] who reported growth improvement in *O. niloticus* when administered with feed fortified with commercial probiotics, Megalo and Diamond-V yeast containing living *Saccharomyces cerevisiae* (*S. cerevisiae*) with *Bacillus subtilis* and dead *S. cerevisiae* respectively. The outcome was probably due to the inhibition of some gut bacterial flora and enhancing the non-specific resistance of the treated *O. niloticus*. Sayed *et al.*[34] further explained that the adherence ability of *S. cerevisiae* and *Bacillus subtilis* to the intestinal mucosa restrained the attachment of the other intestinal bacteria thus, avert occurrence of disease in fish[33].

The result of the gut morphometry of *C. gariepinus* fed with diets fortified with different levels of *L. plantarum* revealed a significant increase ($P < 0.05$) in mean villi length and width compared with the control. This suggests an increase in absorptive surface area of the gut with a resultant increase in body weight gain and feed conversion ratio.

Also, significant increase ($P < 0.05$) was discovered in mean cryptal depth in treated groups which also aid better digestion as suggested by Bowen[35]. This report is similar to the report of some workers who used phytogenic additions to enhance growth of *C. gariepinus*[36,37]. This agrees with final body weight gain, suggesting that 2.0 g probiotic (*L. plantarum*) addition enhanced growth and feed utilization in *C. gariepinus*. Similar outcome was reported by Ghobadi *et al.*[38] where higher feed conversion ratio was observed in the test diets of juvenile common carp (*Cyprinus carpio*) supplemented with Bactocell® additive compared to feed conversion ratio obtained in the control group. The result of gut morphometry in treated group further explained the increased weight gain and feed conversion ratio observed in treated group. Hence, addition of 2.0 g of *L. plantarum* may enhance the growth performance and feed utilization of *C. gariepinus*.

Probiotics have the potential to positively or negatively impact both the animals in aquaculture and the surrounding environment. The characteristics of the bacteria strain and host is very vital and decides the type of interaction and outcome in the bacteria and the host. Therefore, the selection and source of probiotics play an important role. *L. plantarum* isolated from corn slurry could be seen as having growth promoting effect on *C. gariepinus* when supplemented in the feed in order to improve growth performance, nutrient utilization in African catfish. The result of the ninety days feeding trial of *C. gariepinus* fingerlings with diet supplemented with probiotic (*L. plantarum*) revealed that proper and efficient use of *L. plantarum* probiotic as feed additive is valuable for cultured *C. gariepinus* because of its growth promoting effect and nutrient utilization. However growth performance could be enhanced in fish by incorporating probiotics at 2.0 g. Regular application of probiotics through feed to animals reared in captivity can also be used to maintain the microbial population in the gastrointestinal tract at a level that can express sufficient functionality.

There is need for further investigation to thoroughly understand the different methods of interaction of *L. plantarum* isolated from corn slurry under different experimental conditions such as high stocking rate, during infection *etc.* with African catfish with possible protective role against field pathogens.

Conflict of interest statement

We decalre that we have no conflict of interest.

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