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# *Lactobacillus plantarum* Caused Improved Growth and Immunocompetence and Reduced Pathogenic Microbes in Monosex *Oreochromis niloticus*

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## ABSTRACT

**Background and Objective:** The sustainable production of sex-reversed *Oreochromis niloticus* is challenged by disease outbreaks resulting from high stocking densities and indiscriminate use of synthetic drugs in its production. Thus, the probiotic effects of *Lactobacillus plantarum* in sexually reversed male tilapia using growth, serum biochemical and gut microflora indices were evaluated in this study. **Materials and Methods:** Basal diet was supplemented with 0 (Lp0), $1.5\times10^8$ (Lp1), $3.0\times10^8$ (Lp2), or $4.5\times10^8$ CFU mL$^{-1}$ (Lp3) *Lactobacillus plantarum* and fed to triplicate sets of all male *O. niloticus* (2.23±0.04 g) to apparent satiation twice daily for 84 days. **Results:** The higher weight gain (9.56±0.63 g), specific growth rate (1.98±0.05%), protein efficiency ratio (1.61±0.05 g) and superior feed conversion ratio (1.61±0.05 g) in fish fed Lp1 ($p<0.05$) while corresponding least values were observed in Lp0. Histology revealed disseminated steatosis in the liver of all samples including the control. *Lactobacillus plantarum* increased significantly ($p<0.05$) hemoglobin, packed cell volume, white and red cell counts, serum protein, alkaline phosphatase and the levels of alanine aminotransferase (ALT) and aspartate transaminase (AST) were reduced significantly ($p<0.05$) in fish blood. Liver function was improved as shown in the decrease recorded in the measured liver enzyme (AST and ALT) activities. **Conclusion:** The *Lactobacillus plantarum* causes an improvement in the health status and growth of *Oreochromis niloticus*.

## KEYWORDS

*Lactobacillus plantarum*, *Oreochromis niloticus*, growth performance, immunity, gut microbiota, liver



## INTRODUCTION

One of the good candidates for culture in many parts of Africa including Nigeria is the Nile tilapia, *Oreochromis niloticus*, because of its quality flesh and market value but its production is limited due to

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its prolific spawning which results in stunted growth[1]. There have been several efforts at improving tilapia fish production in Nigeria which involves various manipulations such as sex reversal. The sexually reversed *O. niloticus* is an improved strain aimed at ensuring that the metabolic energy is channeled towards growth and improved yield[2], instead of gonadal growth common in stunted un-reversed individuals. However, the intensification of tilapia culture which includes higher stocking densities poses a major challenge. Increased stocking densities of sex-reversed all-male tilapia could induce disease outbreaks resulting in economic loss. This was according to Munguti *et al.*[1] that it is a formidable threat to sustainable tilapia production. More so, the administration of chemotherapeutic agents for disease control has been discouraged or out rightly banned.

Recently, there have been various types of research exploring the potential of probiotics (live microbial feed supplements) as alternatives to chemotherapeutic agents in the treatment of diseases associated with intensive aquaculture practices[3]. According to Gomez-Gil *et al.*[4], probiotics reduce mortalities in cultured species by stimulating the immune system, improving growth and causing an improvement in resistance to infectious diseases. Verschuere *et al.*[5], stated some action pathways of probiotics in aquaculture and these include the production of chemical elements that inhibits the growth of pathogenic bacteria, stimulate the immune response in the host, water quality improvement and enhance the host's nutrition via the production of supplemental digestive enzymes. Lactic acid bacteria including *Lactobacillus* spp., as probiotics are capable of controlling biological processes in aquaculture[6]. This was evident in the report of Ngamkala *et al.*[7], where innate immune response, resistance to *Aeromonas hydrophila* and growth were improved when *Oreochromis niloticus* was fed dietary *Lactobacillus* spp.

Gut morphology and its function are affected by the endogenous microflora in fish. Since the intestinal epithelium can be disrupted by fish pathogens, it follows that a healthy gut microflora will be beneficial in the development of the architecture of gut epithelial. As pointed out by Merrifield *et al.*[8], there is a reduction in mucosal damage, increased absorptive area and disease prevention when the gut microbial population is healthy. Several works have brought to the fore the effect of probiotic use on immunocompetence and endocrine control of growth and appetite in *O. niloticus*[9-11]. However, there is scanty information on the effects of probiotics in mono sex *O. niloticus*. This study thus assesses the influence of dietary *Lactobacillus plantarum* sourced from concentrated starchy liquid from corn, on growth, immune response, gut microflora and histopathology of mono sex *Oreochromis niloticus*.

## MATERIALS AND METHODS

**Study area:** This study was conducted in the wet laboratory, Department of Aquaculture and Fisheries Management fish farm and Fish laboratory of the University of Ibadan. The study was conducted for a period of 84 days (April to June 2017).

**Isolation of probiotic strain and culture condition:** A pure sample of the single-cell proteins (*Lactobacillus plantarum*) was isolated from concentrated starchy liquid (ògì) processed from corn. The isolation of probiotic strain and culture condition follow the procedure of Catherine and Okechi[12].

**Experimental diets:** A basal diet (Lp0) with 30.08% analyzed crude protein content, 6.9% ether extract, 3.6% crude fiber and 7.3% ash content was formulated. Experimental diets were basal diet as control (Lp0), a basal diet supplemented with $1.5\times10^{8}$ CFU mL$^{-1}$ (Lp1), $3.0\times10^{8}$ CFU mL$^{-1}$ (Lp2) and $4.5\times10^{8}$ CFU mL$^{-1}$ (Lp3) (Table 1). The viability test of *Lactobacillus plantarum* (Table 2) in the formulated diets was determined according to Gobi *et al.*[13]. To ensure good viability, diets were prepared every 2 weeks.

**Experimental facility and husbandry:** A total of 360 all-male *O. niloticus* (2.23±0.05 g) was procured and kept for 2 weeks in aerated 1m$^3$ plastic tanks to acclimatize to laboratory conditions[14]. Thereafter, 30 fish each were randomly distributed into twelve hapa (0.5 m$^3$) in three indoor concrete tanks (15 m$^3$), with each

Table 1: Composition (gross) of experimental diets with the probiotic bacterium (*Lactobacillus plantarum*)

| | *Lactobacillus plantarum* | | | |
|---|---|---|---|---|
| Ingredients | Lp0 | Lp1 | Lp2 | Lp3 |
| Fishmeal (72%) (g) | 342.6 | 342.6 | 342.6 | 342.6 |
| Soybean meal (g) | 342.6 | 342.6 | 342.6 | 342.6 |
| Groundnut cake meal (g) | 171.2 | 171.2 | 171.2 | 171.2 |
| Maize (g) | 530.0 | 530.0 | 530.0 | 530.0 |
| Biscuit waste (g) | 530.0 | 530.0 | 530.0 | 530.0 |
| Premix (g) | 10.0 | 10.0 | 10.0 | 10.0 |
| Lysine (g) | 10.0 | 10.0 | 10.0 | 10.0 |
| DCP (g) | 20.0 | 20.0 | 20.0 | 20.0 |
| Methionine (g) | 18.0 | 18.0 | 18.0 | 18.0 |
| Salt (g) | 5.0 | 5.0 | 5.0 | 5.0 |
| *Lactobacillus plantarum* (CFU $g^{-1}$) | - | $1.5\times10^{8}$ | $3.0\times10^{8}$ | $4.5\times10^{8}$ |
| Total | 100 | 100 | 100 | 100 |
| **Analyzed (%)** | | | | |
| Moisture | 9.37 | 9.89 | 9.99 | 10.05 |
| Crude protein | 30.08 | 28.60 | 29.05 | 28.97 |
| Crude fibre | 3.60 | 3.30 | 3.70 | 3.50 |
| Ash | 7.30 | 7.40 | 5.60 | 7.40 |
| Ether extract | 6.90 | 6.70 | 7.10 | 6.60 |

DCP: Dicalcium phosphate

Table 2: Probiotics concentration and viability test

| Diets | Probiotic concentration | Viability test |
|---|---|---|
| Lp1 | $1.5\times10^{8}$ | $1.1\times10^{6}$ |
| Lp2 | $3.0\times10^{8}$ | $2.3\times10^{6}$ |
| Lp3 | $4.5\times10^{8}$ | $2.9\times10^{7}$ |

tank holding four hapas representing replicates of various treatments. Diets Lp0, Lp1, Lp2 and Lp3 were fed to a class of fish in triplicates, two times daily (9 hrs and 16 hrs) to apparent satiation for 12 weeks. Dissolved oxygen, temperature and pH were monitored using LaMotte test kits (AQ-2/AQ-3 Model), while nitrite and ammonia were read using a HACH test kit (FF 1A Model). The quality of these parameters (dissolved oxygen, 4.52±0.21 mg $L^{-1}$, temperature, 26.5±0.25°C, pH, 6.6±0.12, nitrite, 0.15±0.03 mg $L^{-1}$, ammonia, 0.35±0.09 mg $L^{-1}$) throughout the experiment supports the culture of tilapia.

**Estimation of nutrient utilization and growth in *Oreochromis niloticus* fed diets:** Weight changes in fish were measured bi-weekly and feed administered during each regime was recorded and siphoned uneaten feed deducted to determine intake[15]. The recorded values were used to calculate growth and nutrient utilization per treatment according to Akinwole and Faturoti[16].

**Gut bacteria and histopathology analysis:** Prior to the administration of experimental feeds (after acclimatization), two fish were randomly selected from each treatment for gut microflora and histopathology analysis. Also, at the end of weeks 4, 8 and 12, 2 fish per treatment were randomly selected after 24 hrs starvation and the intestine and liver were aseptically removed following the procedure of Opiyo *et al.*[10]. Total viable count of the bacteria (log CFU $g^{-1}$ intestine) was determined using Wincom Colony Counter (16 W, 220 V, 50 Hz). Livers fixed in neutral buffer formalin solution (10%) were taken to the Histology Laboratory, Pathology Department, University College Hospital (UCH), Ibadan. The fixed specimens were prepared routinely. Paraffin sections (thickness 5-micron) were made and stained using hematoxylin and Eosin[17]. Light microscopy (XSZ-07 Series) was used for taking images.

**Blood and serum biochemical indices in *Oreochromis niloticus* fed test diets:** After 84 days of feeding, six fish were selected randomly per treatment and blood samples were collected as described by Ajani *et al.*[18]. Two sets of tubes were used to receive blood samples. Sodium heparinized (20 U $L^{-1}$) tubes received samples for white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb) and packed cell volume (PCV) analysis, while the second set received samples without anticoagulant for total serum protein, alanine transferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) analysis.

**Statistical analysis:** The homogeneity of variances in treatment was tested (Bartlett's test), while the impact of *Lactobacillus plantarum* on fish performance was analyzed using ANOVA (One-Way Analysis of Variance). Treatment means with differences was separated with Duncan Multiple Range Test (DMRT) at a 95% confidence interval using IBM Statistical Package for Social Science (SPSS) version 20.

## RESULTS

Fish growth performance after 12 weeks of feeding was presented in Table 3. Significantly higher ($p<0.05$) final body weight and mean weight gain occurred in *L. plantarum*-fed fish than Lp0 group (control). The SGR and PER in fish-fed Lp0 were lower compared to the *L. plantarum*-treated groups ($p<0.05$). Similarly, FCR was superior in Lp1 ($p<0.05$) (as calculated from the feed consumed and weight gain) and the most inferior value of 2.03±0.03g was recorded in the LP0 fish. The Lp2 and Lp3 groups had statistically similar FCR ($p>0.05$). The survival rate was also statistically similar between treatments ($p>0.05$).

The intestinal bacteria identified in *Oreochromis niloticus* fed *L. plantarum* diets after 84 days were presented in Table 4. *Pseudomonas* spp., *E. coli* and *Klebisiella* spp., were isolated in the guts of all samples at week 4, while *Staphylococcus* spp., was not observed. *Pseudomonas* spp., growth was however more intense in the Lp0 and Lp1 groups at weeks 4 and 8, but reduced in week 12 and was absent in Lp2 and Lp3 at week 12. The *E. coli* was absent in all groups at week 12, while *Kleb* spp., was only observed in fish up to week 4. The quantitative evaluation of bacteria in the guts of fish was presented in Table 5. Total Viable Count (TVC) ranged from 6.04 $\log_{10}$ CFU $mL^{-1}$ in Lp0 to 7.38 $\log_{10}$ CFU $mL^{-1}$ in Lp2. While Lactic Acid Bacteria (LAB) count was highest (6.96 $\log_{10}$ CFU $mL^{-1}$) in Lp2 and least (4.14 $\log_{10}$ CFU $mL^{-1}$) in Lp0. The TVC and LAB were significantly higher in fish-fed *Lactobacillus plantarum* diets.

Histology revealed disseminated steatosis in the liver of all samples including the control group (Fig. 1a). There was congestion in the vessels (Fig. 1b) and infiltration of inflammatory cells in the hepatocyte (Fig. 1c) were observed in the liver of all fish fed probiotic treated diets.

The mean values of haematological and serum biochemical indices of the *O. niloticus* fed *L. plantarum*-based diets are shown in Table 6. All the hematological parameters (RBC, WBC, Hb and PCV) investigated were higher ($p<0.05$) in fish-fed diets with *L. plantarum*. The highest values of RBC (3.05±0.15 g $dL^{-1}$) and PCV (25.15±0.06%) were recorded for Lp1, while WBC (9.24±0.20 $10^6$ $\mu L^{-1}$) and Hb concentration (8.77±0.12 g $dL^{-1}$) show maximum values in Lp3. However, Lp0 had the least values for these parameters.

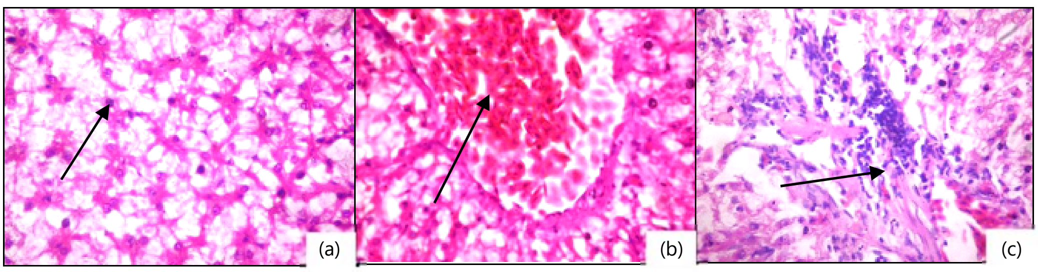


Fig. 1(a-c): Photomicrograph of the histology of the liver of Nile tilapia *Oreochromis niloticus* fed probiotics-based diet, (a) Steatosis in the liver, (b) Congestion of vessel and (c) Focal infiltration of inflammatory cell

Table 3: Nutrients utilization and fish growth fed varying inclusion levels of *L. plantarum*-based diets

| Parameter | Lp0 | Lp1 | Lp2 | Lp3 |
|---|---|---|---|---|
| Initial body weight (g) | 2.24±0.02$^{a}$ | 2.24±0.02$^{a}$ | 2.24±0.04$^{a}$ | 2.21±0.02$^{a}$ |
| Final body weight (g) | 8.61±0.16$^{c}$ | 11.80±0.64$^{a}$ | 9.76±0.19$^{b}$ | 9.47±0.22$^{b}$ |
| MWG (g) | 6.37±0.14$^{c}$ | 9.56±0.63$^{a}$ | 7.52±0.15$^{b}$ | 7.25±0.21$^{b}$ |
| SGR (%) | 1.60±0.01$^{c}$ | 1.98±0.06$^{a}$ | 1.75±0.01$^{b}$ | 1.73±0.02$^{b}$ |
| FCR (g) | 2.03±0.03$^{a}$ | 1.61±0.05$^{c}$ | 1.70±0.00$^{b}$ | 1.78±0.06$^{b}$ |
| PER (g) | 1.63±0.03$^{c}$ | 2.18±0.07$^{a}$ | 2.01±0.02$^{b}$ | 1.97±0.04$^{b}$ |
| Survival (%) | 83.33±0.00$^{a}$ | 83.33±0.00$^{a}$ | 83.33±0.00$^{a}$ | 83.33±0.00$^{a}$ |

Basal diet (Lp0), MWG: Mean weight gain, SGR: Specific growth rate, FCR: Food conversion ratio, PER: Protein efficiency ratio and means with the same superscript on the same row do not vary significantly ($p<0.05$)

Table 4: Microflora isolates from gut samples of *Oreochromis niloticus* fed *Lactobacillus plantarum* diets for 4, 8 and 12 weeks

| Weeks | Treatment | *Pseudomonas* spp. | *E. Coli* | *Staphylococcus* spp. | *Kleb* spp. |
|---|---|---|---|---|---|
| | Lp0 | ++ | + | - | + |
| 4th week | Lp1 | ++ | + | - | + |
| | Lp2 | + | + | - | + |
| | Lp3 | + | + | - | + |
| | Lp0 | ++ | + | - | - |
| 8th week | Lp1 | ++ | + | - | - |
| | Lp2 | + | - | - | - |
| | Lp3 | + | + | - | - |
| | Lp0 | + | - | - | - |
| 12th week | Lp1 | + | - | - | - |
| | Lp2 | - | - | - | - |
| | Lp3 | - | - | - | - |

+: Bacterial growth, ++: More intense growth and -: Absence

Table 5: Bacterial count in the gut of *Oreochromis niloticus* fed *Lactobacillus plantarum* diets

| Treatment | Lactic acid bacterial ($\log_{10}$ CFU mL$^{-1}$) | Total viable count ($\log_{10}$ CFU mL$^{-1}$) |
|---|---|---|
| Lp0 | 4.14$^{c}$ | 6.04$^{b}$ |
| Lp1 | 6.94$^{a}$ | 7.32$^{a}$ |
| Lp2 | 6.96$^{a}$ | 7.38$^{a}$ |
| Lp3 | 6.13$^{b}$ | 7.22$^{a}$ |

Means with same superscript on same row do not vary significantly ($p<0.05$)

Table 6: Blood and biochemical parameters in *Oreochromis niloticus* fed varying inclusion levels of *L. plantarum* based diets for 84 days

| Parameter | Lp0 | Lp1 | Lp2 | Lp3 |
|---|---|---|---|---|
| Red blood cell (g dL$^{-1}$) | 2.22±0.5$^{d}$ | 3.05±0.15$^{a}$ | 2.59±0.11$^{c}$ | 2.85±0.08$^{b}$ |
| White blood cell ($\times10^{6}$ µL$^{-1}$) | 8.03±0.46$^{c}$ | 8.80±0.55$^{b}$ | 9.11±0.28$^{a}$ | 9.24±0.20$^{a}$ |
| Hemoglobin (g dL$^{-1}$) | 6.98±0.07$^{c}$ | 8.24±0.10$^{b}$ | 8.51±0.05$^{ab}$ | 8.77±0.12$^{a}$ |
| Packed cell volume (%) | 21.39±0.04$^{d}$ | 25.15±0.06$^{a}$ | 23.56±0.03$^{c}$ | 24.77±0.07$^{b}$ |
| Total protein (g dL$^{-1}$) | 3.09±0.03$^{a}$ | 3.42±0.10$^{a}$ | 3.50±0.07$^{a}$ | 3.17±0.08$^{a}$ |
| AST (U L$^{-1}$) | 64.62±0.13$^{a}$ | 52.14±0.05$^{b}$ | 58.05±0.18$^{b}$ | 45.56±0.08$^{c}$ |
| ALT (U L$^{-1}$) | 35.39±0.11$^{a}$ | 24.10±0.06$^{c}$ | 23.71±0.12$^{c}$ | 29.67±0.18$^{b}$ |
| ALP (U L$^{-1}$) | 21.28±0.08$^{c}$ | 24.23±0.11$^{a}$ | 23.88±0.05$^{a}$ | 22.75±0.06$^{b}$ |

Means with the same superscript on the same row do not vary significantly ($p<0.05$), AST: Aspartate aminotransferase, ALT: Alanine aminotransferase and ALP: Alkaline phosphatase

Further, fish fed probiotic treated diets exhibited slightly higher total serum protein levels ($p>0.05$) compared to Lp0. Also, there was a noticeable decrease in transferase enzymes (ALT and AST) in the *L. plantarum* treated groups when compared with the control. The Lp1 and Lp2 fish had a signi cant higher and lower concentrations of AST and ALT, respectively. Significantly higher ALP levels were recorded in groups fed *L. plantarum* compared to the Lp0 group. Tilapia fed $1.5\times10^{8}$ CFU g$^{-1}$ and $3.0\times10^{8}$ CFU g$^{-1}$ *L. plantarum* treated diets showed significantly the highest levels of ALP ($p<0.05$).

## DISCUSSION

*Lactobacillus plantarum* resulted in growth and nutrient utilization improvement in fingerlings of mono-sex Nile tilapia. Fish-fed *L. plantarum* exhibited higher body weight gain, rate of growth and protein efficiency ratio than the control groups. The best growth was recorded in fish fed 1.5 g $kg^{-1}$ *L. plantarum*-based diet and a non-linear relationship was observed between probiotic inclusion level and growth. Improvement of feed utilization and growth in groups fed *L. plantarum* supplemented diets may be a result of an improvement in the balance of the intestinal microbial population which in turn led to enhanced digestibility of nutrients, more enzyme activities and improved nutrient absorption. This was in agreement with Essa *et al.*[19], where significantly higher growth performance was reported in *O. niloticus* fed to *Lactobacillus plantarum*, Mohapatra *et al.*[20], when fed to *Labeo rohita*. Similarly, growth and feed efficiency were reported to be higher in *Oreochromis niloticus* fed *L. plantarum*-based diets compared to the control group[21].

A significantly higher feed conversion ratio was observed in Lp0 fish when compared to those fed probiotic diets in this study. And this bespeaks that, fish in the control consumed more feed without a corresponding conversion of same to the flesh. Supplementation of probiotics in diets of fish was reported to better the conversion of feed to flesh via improved utilization of nutrients[11]. The experimental diets in this study did not result in any significant variation in the rate of survival of mono-sex tilapia, implying that the nutritional requirements were met.

Intestinal microbes' population modulation represents one of the established modes of action of these live microorganisms in the host. According to Lazado and Caipang[22], this action influences disease, nutrition, growth and immunity. In this present study, the major microbiota isolated in the intestine of *Oreochromis niloticus* after 12 weeks of feeding trial includes *Pseudomonas* spp., in the control and Lp1 groups, while no bacteria were isolated in the other treatments at this time. This may be attributed to the dominance of the Lactic acid bacteria at week twelve as shown in the result of the quantitative evaluation of gut bacteria. *Pseudomonas* spp., was predominant in all treatments up till week 8 in this present study. *Pseudomonas* spp., has been reported as the most common genera in the fish intestine[23]. The composition of the microbes in the intestine is similar to previous reports on Nile tilapia[24,25], although with varying levels of abundance.

During the 12th week, *Pseudomonas* spp., was only isolated in all Lp0 and Lp1 while other no other bacteria was isolated in any groups. This result suggests the modulation of the gut microbes by *L. plantarum* which is well established in fish guts after 84 days of feeding. Falcinelli *et al.*[26], reported that the addition of probiotics to fish feed is linked with changes in gut microbes and these changes modulate the manifestation of a complex genes network. In some fish, modulation results in increased glycemia and elimination or reduction of some gut flora, thus accounting for the differences observed in the bacteria counts of fish fed varying levels of *L. plantarum*.

The liver is the main fish metabolic organ that plays roles in toxins ingestion, accumulation, bioconversion and excretion[27]. In this present study, however, the disseminated steatosis in all fish (both control and treated) showed that *L. plantarum* may not be associated with abnormal retention of triglycerides within the liver cells. The inflammation of the hepatocytes may be attributed to the direct breaking of bonds in enzymes that regulate the volume or indirect distortion of ionic regulation due to disruption of energy transfer in cells[28]. The histological changes identified in the liver as analyzed were not linked to the supplementation of diets with *L. plantarum*, however, further studies are recommended to ascertain this.

Physiological changes in fish resulting from stress conditions are studied using hematological indices. In this present work, better profiles were observed in blood samples from fish-fed *L. plantarum* diets, with significantly higher values of RBC, PCV, WBC and Hb compared to Lp0. This result agrees with previous observations of improved blood profile in *Oreochromis niloticus* fed probiotic-treated diets[9,10]. Fish fed a $1.5\times10^8$ CFU $g^{-1}$ *L. plantarum* diet recorded higher levels of RBC and PCV, while high concentrations of

WBC and Hb were observed in the $4.5\times10^8$ CFU $g^{-1}$ *L. plantarum* group. This result suggests improved immunity in fish fed *L. planetarium* as additive[29]. In this present work, a significant reduction in the activity of liver enzymes (ALT and AST) was observed in fish-fed diets fortified with *L. plantarum* compared to the Lp0 fish. These results indicate that the liver was not stressed as the fish had a better health status. This submission is supported by Omitoyin *et al.*[14], where a reduction in the levels of ALT and AST in the liver of *O. niloticus* fed photogenic extract was attributed to the protection of the liver's membrane integrity by it. However, this result contradicts the findings of Won *et al.*[11], who reported that liver enzyme (AST and ALT) activity was not a ected by probiotic supplementations in Nile tilapia fingerlings. Also, increased ALP level in probiotic-treated diets shows better absorption in the body of the fish. According to Omitoyin *et al.*[14], increased ALP shows liver cells are functioning properly.

The present study showed a significant contribution of dietary *L. plantarum* $1.5\times10^8$ CFU $mL^{-1}$ to maximum growth, feed utilization efficiency and hematology of *O. niloticus* fingerlings. This may be attributed to the higher ability of *Lactobacillus* sp., to mitigate the effects of the stress factors, which resulted in better growth performance. However, further research is needed to determine the stimulatory effect of dietary *L. plantarum* on immune parameters and resistance of *O. niloticus* to infectious pathogens.

## CONCLUSION

In conclusion, the results of the present study indicated that supplementation of *Lactobacillus plantarum* at $1.5\times10^8$ CFU $mL^{-1}$ basal diet enhanced the growth performance of *Oreochromis niloticus*. The *L. plantarum*-based diet showed the capacity to regulate blood cells including red and white blood cells, without having effects on the liver structure and function.

## SIGNIFICANCE STATEMENT

This study discovered that *Lactobacillus plantarum*-based diets can be beneficial in the production of Nile tilapia and *Oreochromis niloticus* with improvement in growth, health and immune system. This study will help the researchers to uncover the critical areas of ideal inclusion level for improved feed efficiency that many researchers were not able to explore. Thus, a new theory on probiotics from Lactic acid bacteria may be arrived at.

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