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Improvement of the Growth Performance, Innate Immunity and Disease Resistance of Nile Tilapia (*Oreochromis niloticus*) against *Vibrio parahaemolyticus* 1T1 following Dietary Application of the Probiotic Strain *Lactobacillus plantarum* 1KMT

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Authors' contributions

This work was carried out in collaboration among all authors. Author TJB designed the study, wrote the protocol, performed the experimental analyses and wrote the first draft of the manuscript. Authors KPM and ZNF managed the literature searches and the analyses of the study. Authors MAG and RK provided equipment, reagents and analyses the study. All authors read and approved the final manuscript.

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ABSTRACT

The impact of *Lactobacillus plantarum* 1KMT on the survival rate, growth performance, innate immunity and disease resistance of *Oreochromis niloticus* challenged with pathogenic *Vibrio parahaemolyticus* 1T1 was investigated. This study was conducted in the Fisheries Department of

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National Agriculture Research Centre (NARC), and Department of Biosciences of Comsats University Islamabad, Pakistan between August 2018 and August 2019. Sixty healthy fish (10.99 ± 1.1 g body weight) were acclimatised to laboratory conditions in 1000 L tank for 14 days. After, they were randomly divided equally into two groups G1 and G2 with one replication: G1 fed with the basal diet (BD) without probiotic (control), and group G2 was fed with $1x10^8$ $CFU.g^{-1}$ *Lactobacillus plantarum* 1KMT supplemented diet. The trial was conducted for a period of 60 days. The intestinal microflora, survival rate (SR), weight gain (WG), specific growth rate (SGR) and food conversion ratio (FCR) were checked after every ten days. After 60 days, the innate immune parameter levels were evaluated. For challenge study against *Vibrio parahaemolyticus* 1T1, eighteen fish from G2 were randomly selected and divided in two subgroups: G2A, with 1KMT continuous treatment and G2B in which treatment was stopped. After 21 days, the mortalities were recorded. The results showed that the intestinal microflora was significantly improved in G2 compared to G1. The survival rate was 96.5% and 86.0% in G2 and G1, respectively. The WG was not significantly affected, while the SGR and innate immunity parameters were significantly improved ($p < 0.05$) in G2 compared to G1. The mortality rate was 77.7% and 66.6% in G1 and subgroup G2B, respectively, while no mortality was observed in G2A after the challenge test. The *Lactobacillus plantarum* 1KMT improves the growth performance, survival rate, innate immunity and disease resistance of Nile tilapia.

Keywords: Aquaculture; Oreochromis niloticus; probiotic; Lactobacillus plantarum 1KMT; infection.

1. INTRODUCTION

Aquaculture has become one of the most rapidly evolving activities in the world. The major importance of aquaculture (for fish) is to cover the population's need for food, especially high-quality animal protein, to generate employment and income [1]. However, fish production today is very inadequate to meet the growing demand, due to the continuous and rapid increase of the world population [2]. Moreover, according to the Food and Agriculture Organisation estimation, the world population to feed would be 9 billion by 2050, hence the need to increase production [2]. In Cameroon, people consume fish mostly than other animal's protein. But, national fish production is very low, about 180 000 tons per year with less than 1000 tons from aquaculture compared to 400 000 tons population's demand [3]. The government spend a lot of money every year for fish importation to fill 212000 tons gap [4]. This has led to the intensification of aquaculture to solve this problem. However, it is well recognized that intensive aquaculture suffers from bacterial infection problems. Several previous studies have already revealed cases of infections due to pathogen bacteria. For example, *Vibrio*, specifically *Vibrio parahaemolyticus* is one of the pathogenic bacteria that causes diseases and losses in aquaculture. Its physiological characteristics (high salt tolerance, growth temperature between 10 and 45° C, low nutrient demand) allow it to grow rapidly in most aquatic environments [5]. *Vibrio parahaemolyticus* is responsible for acute hepatopancreatic necrosis disease (AHPND), characterised by loss of appetite and equilibrium, abdominal haemorrhages and dark color in fish [6]. Other microorganisms such as *Salmonella* spp. [7], *Staphylococcus* spp. [8], *Pseudomonas aeruginosa* [9], *Aeromonas hydrophila* [10], *Listeria monocytogenes*, and *Clostridium botulinum* [11] are also responsible for many infections in aquaculture. To prevent and solve these problems of infections, most fish farmers use daily chemical compounds such as antibiotics [12]. The abusive, hazardous and sometimes uncontrolled use of these chemicals leads with the time to serious problems such as drugs resistance, pollution of the aquatic environment [13,14] and residue related issues [15]. However, the persistence of these infections leads to the introduction of probiotics in aquaculture sector as alternatives to antibiotics [16].

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [17]. Upto now, probiotics in animals has not yet shown any negative effects [18]. On the contrary, they act through several mechanisms such as the production of some digestives enzymes to increase food digestion and nutrients absorption [19]. Probiotics play also an important role to prevent and control pathogens invasion by occupying the fixation sites, and by producing the antimicrobial compounds at the same time in the intestinal mucosa. They are also used as growth promoters and immunomodulators in animals

such as fish [20]. Moreover, several studies reported utilisation of probiotic lactic acid bacteria in aquaculture. In this regard, the probiotic lactic acid bacteria *Bacillus subtilis* [20,21,22] and *Bacillus licheniformis* [23] have already shown their beneficial effects on growth performance, immune response and disease resistance in Nile tilapia *Oreochromis niloticus* and *Cyprinus carpio*. Intensification of aquaculture to fill the current deficit remains a challenge. Among the countries recognized as major fish producers, Egypt remains the only African country where intensive aquaculture is practised [2]. Some studies in this country showed also that probiotic *Bacillus subtilis* at different concentrations can improve growth performance and immune response in Red Seabream [24]. However, the aquaculture environment of most sub-Saharan countries showed rarity of probiotic lactic acid bacteria strains adapted to tropical ecological conditions. Nevertheless, in our previous studies [25], the strain *Lactobacillus plantarum* 1KMT isolated from the gut contents of Nile tilapia showed interesting probiotic potential *in-vitro*. Indeed, this strain demonstrate high ability of auto-aggregation and co-aggregation [25], high antimicrobial activity against many spoilage and pathogens bacteria [26]. *Lactobacillus plantarum* 1KMT showed also no resistance to antibiotics, hemolytic and gelatinase activities, or biogenic amines production [25]. However, this probiotic has not yet been used in aquaculture to evaluate their effects in-vivo. Thus, this study aimed to evaluate the impact of *Lactobacillus plantarum* 1KMT to act under real conditions on the growth performance, innate immunity parameters and disease resistance of Nile tilapia (*Oreochromis niloticus*) to *Vibrio parahaemolyticus* 1T1 infection.

2. MATERIALS AND METHODS

2.1 Probiotic Strain and Preparation of Probiotic Supplemented Diet

Probiotic strain *Lactobacillus plantarum* 1KMT, used in this study was isolated from the gut content of *Oreochromis niloticus*, identified and its probiotics properties were evaluated *in – vitro* in our previous studies [25,26]. To obtain the probiotic strain suspension, *Lactobacillus plantarum* 1KMT was activated in MRS broth (Oxoïd Ltd, Basingstoke, Hampshire, England), incubated for 24 h at 30°C. The cells were harvested by centrifugation (4000 rpm/min) at 4°C for 20 min, washed twice with sterilised Phosphate Buffer Saline (PBS, pH 7.2), and re-suspended in the PBS. The turbidity was compared to the 0.5 McFarland standard solution to obtain 1 x 10^8 CFU/mL [27]. Before adding the probiotic strain, the basal diet (BD) was sterilised in the oven at 100°C for 24 h to eliminate the possible presence of other microorganisms that could interfere with the probiotic activity. Then, the required amount (1 mL for 20 g) [28] of probiotic suspension was sprayed into the diet slowly, and mixing under sterile conditions. The diet was dried in oven at 30°C for 5 h and stored at – 20°C until used. The viability of *Lactobacillus plantarum* 1KMT in the BD was investigated at 0, 3, 6 and 9 days of storage, using enumeration method on solid MRS. *Lactobacillus plantarum* 1KMT level decreased by 10% after 8 days. Therefore, fresh diet was prepared accordingly after 7 days to ensure high probiotic level in the diet.

2.2 Fish and Rearing Conditions

Sixty healthy fish *O. niloticus* (bodyweight 10.99 ± 1.1 g) collected from Fisheries Department of National Agricultural Research Centre (NARC), Islamabad-Pakistan, were stipulated into 1000 L indoor tanks for 14 days acclimatisation to the laboratory conditions, and fed with basal diet, formulated from locally available ingredients (Table 1). The proximate composition of feed was determined by the AOAC (Association of Official Analytical Chemists) method [29], and was consisting to 89.98% dry matter, 31% crude lipid and 29.84% crude protein. Afterwards, the fish were randomly divided into two equal groups: G1 (control) and G2 with one replication (four aquariums in total with 15 fish in each). The group G1 was fed with the BD. The G2 group was fed with 1 x 10^8 CFU.g^{-1} *Lactobacillus plantarum* 1KMT supplemented diet. The dose was selected based upon the results from previous studies [27]. Fish were fed twice daily (10 am and 4 pm) at 3% of the bodyweight [30] for 60 days.

To ensure the hygienic conditions during the trial, the water quality parameters were checked daily, and 30% of the water of each aquarium was exchanged every day and the whole was renewed after every 7 days. The temperature was controlled (22.4 ± 1.37°C), pH (7.79 ± 0.4), and the water dissolved oxygen remained within a range of 6.63 – 7.56 mg L^{-1} during the whole experiment. To determine the effect of probiotic strains on the intestinal microflora, the fish faeces were collected from each group after every 10 days and the microbial load of

Salmonella sp., *Vibrio* sp., *Staphylococcus* sp., *Pseudomonas* sp., *E. coli* and lactic acid bacteria, respectively were evaluated by enumeration on Salmonella–Shigella Agar (SSA, Liofilchem), TCBS (BIOCHEM Chemopharma), Mannitol Salt Agar (MSA, Biochem Chemopharma), King A (Scharlau), Violet red bile dextrose agar (VRBDA, Scharlau) supplemented with 4-methyl-umbeliferil-β-D glucuronide (MUG), and MRS (Oxoïd Ltd, Basingstoke, Hampshire, England) culture medium, respectively. Briefly, 1 g of faeces collected from each group was diluted in 9 mL of sterile physiological solution (0.9% NaCl) and decimal dilutions were made. Then 100 microliters of each dilution were spread on the surface in petri dishes containing the specific culture medium for each bacterium tested. Petri dishes were then incubated at 30°C for lactic acid bacteria and 37°C for other bacteria.

2.3 Growth Performance and Survival Rate

At the beginning of the experiment, the weight and size of each fish were measured, and the total biomass in each aquarium was evaluated. The growth performance was evaluated every ten days and the amount of feed was adjusted accordingly. In the end, all fish from each aquarium were collected, the total number of fish was noted for calculating the average weight and size. The growth performance of fish was evaluated in terms of weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and survival rate according to the formula [31]:

- WG (g) = Final weight – Initial weight
- SGR (%.day^{-1}) = ((Ln final weight – Ln initial weight)/number of days) x 100
- FCR = Total feed intake/Total weight gain
- Survival rate (%) = (Number of fish survived after 60 days/Initial number of fish stocked) x 100.

2.4 Blood Samples Collection

Blood samples were collected according to the method described by Giri et al. [27] with slight modifications. After 60 days of the feeding trial, blood samples were collected from the caudal vein of six fish per aquarium (12 fish per treatment) using 2 mL syringe after removal of the tail with a sharp and sterile knife. The fish were anesthetised with ethanol 90° and the samples were collected separately: one part of the blood was dropped into the EDTA tubes to prevent coagulation. The whole blood was used directly to check the haematological parameters. Plasma was then separated by centrifugation at 4000 x g for 15 min and stored at -20°C. Another set of blood sample of the same fish was collected into the dry tube without EDTA and allowed to clot at room temperature for 30 min. The serum was then separated by centrifugation at 4000 x g for 15 min and stored at -20°C for analyses of innate immune parameters.

2.5 Haematological Parameters

The blood samples collected previously into the EDTA tubes were used immediately for analysis. Ullah et al. [32] method with few modifications was adopted for estimating the complete blood count (CBC) such as Red blood cells (RBC), haemoglobin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets were taken using automatic Haematology Analyser (BECKMAN COULTER A^{c}-T, Germany). The white blood cells (WBCs) was determined by manual enumeration using a haematocytometer. 10 µL of blood was diluted in 190 µL of dye coloured white blood cells. Then, 2 µL of the final solution was spread on the haematocytometer and the number of white blood cells was counted by optical microscope observation (G x 40).

2.6 Innate Immune Parameters

2.6.1 Serum bactericidal activity test

Serum bactericidal activity was determined following the method described by Barnes et al. [33] with modifications. Fresh pure culture of *Vibrio parahaemolyticus* 1T1 was adjusted to 1x10^{8} CFU/mL using 0.5 Mcfarland standard. Bacterial suspension and serum samples were mixed with 0.5:0.5 ratio and incubated for 90 min at 37° C. The mixture was diluted with Lysogeny Broth (LB) and plated on LB plates to culture for 24 h at 37° C. PBS was used as a negative control. Viable colonies were counted and results were shown as survival percentage of bacterial colonies compared with the negative control.

2.6.2 Lysozyme activity test

The serum lysozyme activity was determined using a turbidimetric assay by the Binuramesh et al. [34] method with modifications. On a flat-

bottomed 96-well microtitre plate, 100 µL of suspension of *Vibrio parahaemolyticus* 1T1 in 0.05 mol.L^{-1} PBS (pH 5.2) was added to 100 µL of the serum, and homogenised immediately. The optical density (OD) reading was taken at 570 nm using a spectrophotometer at 0, 15, 30, 45 and 60 min. A unit of lysozyme activity was defined as the sample amount causing a decrease in absorbance of 0.001 min^{-1}. Lysozyme activity was expressed in lysozyme concentration by taking hen egg-white lysozyme (Sigma, USA) as a standard.

Table 1. Composition of basal diet from local ingredients

Ingredients	Quantities (for 100 Kg)
Fish meal	30
Soybean meal	13
Sunflower meal	5
Canola seed meal	5
Rice polish	17
Gluten 30%	13
Wheat bran	13
Vitamin C	0,5
Vitamin premix	1,5
Soybean oil	2
Total	100

2.6.3 Complement activity test

Complement activity was tested using sheep red blood cells (sRBC) as a target according to the method described by Mohammadian et al. [35]. The sRBC were diluted in PBS (0.1 M, pH 7), containing 75 µL of $MgCl_2$, 1M and 150 µL of $CaCl_2$, 1M. The cells were then washed by centrifugation at 750 rpm for 5 minutes, then resuspended in PBS, and the cell concentration was adjusted to $1x10^8$ cells mL^{-1}. 30 µL of this suspension was mixed with 12 mL of 1.5% agarose and then poured into each petri dish. After solidification, 3 mm wells were dug, then filled with 20 µL of each serum sample and incubated at room temperature for 48 h. After this time, the diameters of the lysis were measured and expressed in mm.

2.6.4 Serum IgM level

The serum IgM level was measured with the ELISA (Enzyme-linked Immunosorbent Assay) assay using a specific commercial Kit for fish IgM (Bioassay Technology Laboratory, Yangpu Dist. Shanghai, China) following the method described by the manufacturers. The ELISA plates were read at 450 nm using an automatic microplate reader (Pro-Reader 96, GMP V650.1.8.0601).

2.6.5 Challenge study

The challenge study was performed according to the method described by Giri et al. [27] with modifications. *Vibrio* sp. 1T1 was grown in nutrient broth at 37° C for 24 h. The culture was centrifuged at 4000 rpm for 15 min, and the pellet was washed twice and resuspended in sterilised PBS (50 mM, pH 6.8). After 60 days of trial, eighteen fish were randomly collected from group G2 and divided in two subgroups G2A and G2B (nine fish in each aquarium). Subgroup G2A still receiving probiotic treatment, while the treatment was stopped in subgroup G2B. All fish received by intraperitoneal injection 100 µL [36] of 10^5 CFU/mL of the pathogenic strain *Vibrio parahaemolyticus* 1T1. Nine fish from G1 (control) were also collected and used for challenge study. The mortality rate was determined up to 21 days of challenge study.

2.7 Statistical Analyses

The results are expressed as mean ± standard deviation. One-way ANOVA was used. To determine whether if the difference is significant between variables, the Tukey-Kramer multiple comparison test was used to evaluate the different level. The difference was considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Variation of the Microbial Load during the Experiment

The results of the microbiological analysis are showed on Figs. 1 and 2 for G1 and G2, respectively. From Fig. 1, we observed that the lactic acid bacteria load was lower than other microorganisms load. This result was similar to those obtained by Papadopoulos et al. [37] who reported in their study that under normal conditions, lactic acid bacteria are dominated by *Vibrionaceae* and *enterobacteriaceae*. However, in Fig. 2, whose fish was fed with *Lactobacillus plantarum* 1KMT supplemented diet, a progressive decrease in loads of *Salmonella* sp., *Vibrio* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *E. coli* was observed over time (from day 3 and day 10 in G2), and an increase of lactic

acid bacteria load in the same group. This means that the probiotic *Lactobacillus plantarum* 1KMT has a positive effect on other microorganisms. This positive effect can be due to the production of antimicrobial compounds such as organic acids and others. In fact, *Lactobacillus plantarum* 1KMT produced a large amount of lactic, propionic, valeric and butyric acids, which inhibit different pathogenic microorganisms [26]. Others studies reported that several species of *Lactobacillus* produce bactericidal proteins (bacteriocin) and other compounds which exhibit strong antimicrobial activity against several pathogenic microorganisms [38,39].

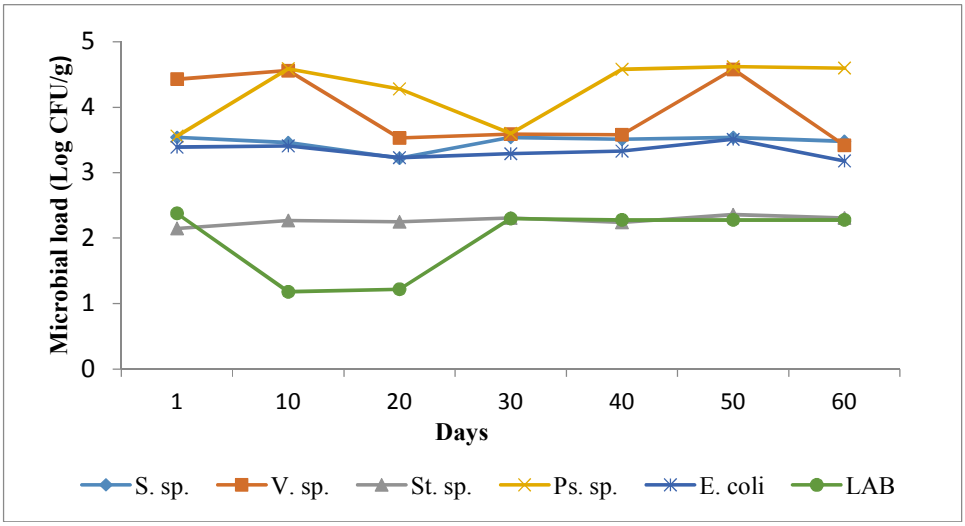

Fig. 1. Variation of the microbial load of fish faeces in group G1 (control)
S.: Salmonella; V.: Vibrio; St.: Staphylococcus; Ps.: Pseudomonas; E.: Escherichia; LAB: Lactic Acid Bacteria

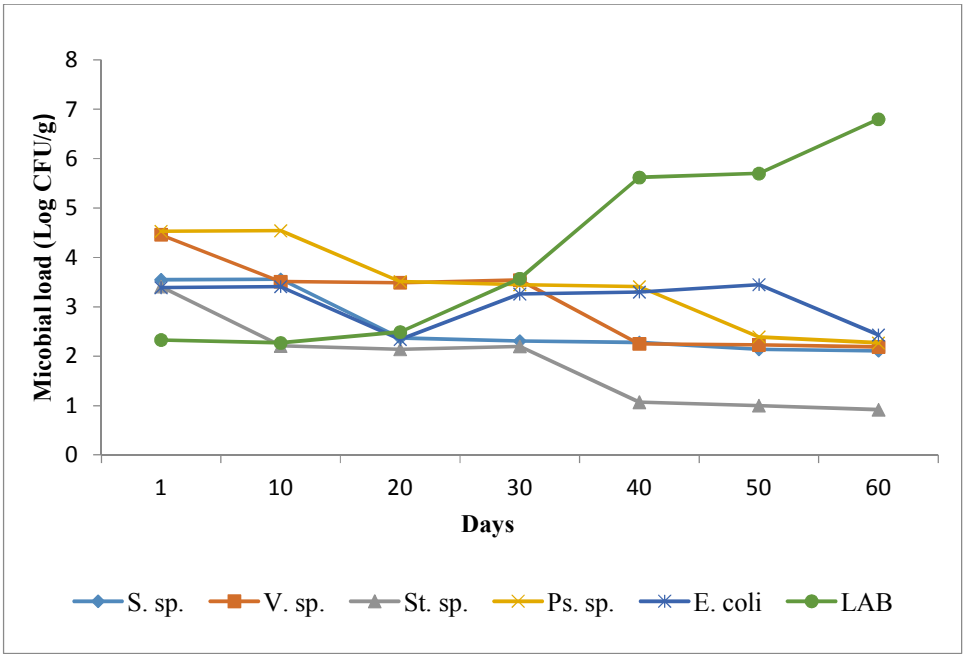

Fig. 2. Variation of the microbial load of fish faeces in group G2 fed with *Lactobacillus plantarum* 1KMT supplemented diet
S.: Salmonella; V.: Vibrio; St.: Staphylococcus; Ps.: Pseudomonas; E.: Escherichia; LAB: Lactic Acid Bacteria

3.2 Growth Performance and Survival Rate

Growth parameters of *Oreochromis niloticus* are summarized in Table 2. The survival rate observed at the end of the experiment was significantly different ($p < 0.05$) in G2 fed with *Lactobacillus plantarum* 1KMT supplemented diet, compared to control group G1. This high rate can be explained by the adaptation of fish to different diets used during rearing. Similarly, the high survival rate observed in G2 fed with *Lactobacillus plantarum* 1KMT reflects the safety (non-toxicity) of this probiotic used in this study. However, some dead fish were counted in these two groups G1 and G2 although no signs of infection were observed. These mortality cases could probably be due to stress related to the operations of taking biological parameters (weight and size) and cleaning aquariums following the jump of fish outside the aquariums during the trial. These results are similar to those obtained by Chemlal [40] which reported a survival rate ranging from 96.66% to 100% using probiotic strains in fry *Oreochromis niloticus*. Statistical analysis of the final body weight showed that the difference was not significant ($p > 0.05$) between G1 and G2 values, but the high value was observed in G2. The higher weight gain and specific growth rate were observed in G2 compared to G1 (control). These results could be explained by the fact that *Lactobacillus plantarum* 1KMT used in this study have a positive influence on weight gain in these fish. Indeed, several studies already showed that probiotics can produce or stimulate digestive enzymes production. Bairagi et al. [41] reported in their studies the extracellular production of proteases, amylases and cellulases by some strains of *Bacillus* spp., which increases feed digestibility, thus facilitating nutrients absorption and their storage in the tissues. Ullah et al. [32] also reported in their studies increasing of proteases, amylases and cellulases activities in Moru (*Cirrhinus mrigala*) fed with commercial probiotic supplemented diet. Similar results have previously been reported using probiotics on other fish species such as *Labeo rohita* [42,43] and *Oreochromis niloticus* [40]. Fish size was also improved in Group 2 compared to Group 1. However, no significant difference was observed after the statistical analyses. Specific growth rate (SGR) and food conversion ratio were greatly improved in group 2 fed with the *Lactobacillus plantarum* 1KMT supplemented diet. This result was similar to those obtained by Chemlal [40] in which SGR and FCR were increased in *Oreochromis niloticus* after probiotic treatment.

3.3 Haematological Parameters

The results of the haematological parameters are presented in Table 3. They showed that the platelets, RBC, Hb and MCH levels were not significantly ($p < 0.05$) affected after treatment with the probiotic strain *Lactobacillus plantarum* 1KMT in G2. In agreement with our results, Iwashita et al. [44] reported no changes of some blood parameters like hemoglobin level in tilapia fed diets supplemented with *Bacillus subtilis*. Significant improvement in the Mean corpuscular hemoglobin concentration (MCHC) and hematocrit (Hct) of *Oreochromis niloticus* were reported in this study by *Lactobacillus plantarum* 1KMT supplementation. Similar to our results, Zaineldin et al. [24] and Telli et al. [45] reported increased levels in red sea bream and Nile tilapia, respectively by *Bacillus subtilis* supplementation.

Table 2. Growth performance of Nile tilapia fed with *Lb. plantarum* 1KMT supplemented diet

Parameters	G 1 (Control)	G 2
Survival rate (%)	86.00 ± 3.50^{a}	96.50 ± 3.50^{b}
Initial body weight (g)	10.99 ± 1.10^{a}	10.85 ± 0.99^{a}
Final body weight (g)	23.37 ± 4.64^{a}	25.38 ± 3.14^{a}
Weight gain (g)	12.38 ± 3.54^{a}	15.28 ± 2.15^{a}
Initial biomass (g)	$164.85 \pm 0,54^{a}$	162.75 ± 0.80^{b}
Final biomass (g)	339.03 ± 15.27^{a}	380.80 ± 0.89^{b}
Initial size (cm)	7.68 ± 0.30^{a}	7.45 ± 0.15^{a}
Final size (cm)	10.69 ± 0.94^{a}	11.06 ± 0.70^{a}
SGR (%.day^{-1})	0.13 ± 0.05^{a}	0.33 ± 0.10^{b}
FCR (g.J^{-1})	3.02 ± 0.05^{a}	2.73 ± 0.02^{b}

G1: Control (without probiotic); G2: Group fed with Lactobacillus plantarum 1KMT supplemented diet. Values with different letters on the same line differ significantly ($p < 0.05$); Values are presented as the mean ± S.D

Table 3. Haematological parameters of fresh blood collected after 60 days of experiment in *Oreochromis niloticus*

Groups	Haematological parameters							
	WBC	**RBC**	**Hb**	**MCH**	**MCV**	**MCHC**	**Hct**	**Plt**
G1 (Control)	5.03±0.35[a]	0.63±0.40[a]	3.8±2.26[a]	67.33±11.46[a]	+++++	----	----	68.66±30.56[a]
G2	4.63±0.27[a]	0.54±0.02[a]	3.36±0.09[a]	61.36±2.61[a]	145.15±3.15	42.02±1.25	7.75±0.25	84.66±35.59[a]

Results are expressed as mean ± SD; n = 4. Mean value in the same column with different superscript letter indicate significant ($p < 0.05$) different. +: Very high value; -: Very low value. WBC: White blood cells, RBC: Red blood cells, Hb: Hemoglobin, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Hct: Hematocrit, Plt: Platelets

3.4 Innate Immunity Parameters after 60 Days of Feeding

The results of serum bactericidal, lysozyme, complement activities, as well as serum IgM level are summarised in Table 4. After sixty days of trial, all these parameters were significantly increased ($p < 0.05$) in G2 fed with *Lactobacillus plantarum* 1KMT supplemented diet, compared to control group G1. This means that probiotic *Lactobacillus plantarum* 1KMT act positively on the innate immune parameters of *Oreochromis niloticus*. Several recent studies also showed the improvement of the immune system by probiotic strains in different fish species. Mohammadian et al. [46] showed in their studies that probiotic strain *Lactobacillus casei* significantly increased serum bactericidal, lysozyme and complement activities. Lysozyme and complement are enzymes that act on the membrane of bacteria by breaking the beta-1,4 bond between the N-Acetylmuraminic and N-acetylglucosamine of peptidoglycan (case of lysozyme) and by forming membrane pores by polymerisation (case of complement), and thus promoting their destruction [47]. This could explain the resistance of fish after pathogen infection. Previous studies have already reported the improvement of the immune response in different fish species [48,49,50,51,52]. In case of infection, IgM is mainly produced and is also the most abundant in the blood circulation [53]. In our study, the serum IgM level was significantly increased in G2 (770.87 ± 21.69 µg/mL) group treated with probiotic *Lactobacillus plantarum* 1KMT, compared to G1 (control) (705.62 ± 15.3 µg/mL). These results are similar to those of several previous authors who showed in their study the improvement of serum IgM production by probiotics. Giri et al. [27] showed in their study a strong increase in serum IgM concentration in *Labeo rohita* fed with the probiotic strain *Pseudomonas aeruginosa* VGS-2. Nandy et al. [54] also reported in their study increasing in serum IgM concentration before and after the challenge-test with the pathogenic bacteria *Aeromonas hydrophyla* using a probiotic *Bacillus* sp. in *Labeo rohita*. Recently, Surintorn et al. [55] demonstrated in their study that 10^6 - 10^8 CFU/g of *Saccharomyces cerevisiae* supplemented diet increased the production of several immune parameters including IgM in catfish *Pangasianodon hypophthalmus*. However, serum IgM concentration varies considerably depending on the species, physiological conditions (stress, health status) and environmental conditions such as temperature and water quality, because these parameters change considerably from one environment to another [56]. In this study, fish were subjected to the same rearing conditions, and the only variable was the presence of the probiotic strain in G2, this means that this probiotic strain would have an immunomodulatory effect on the IgM production, although the mechanism is not yet very well clarified [57].

3.5 Challenge Study

After 21 days of challenge, the mortality rate was recorded and summarised in Table 5. It showed 77.7% in G1 without treatment, 66.6% in subgroup G2B in which treatment with probiotic was stopped before challenge test, and 0% in subgroup G2A in which treatment with *Lactobacillus plantarum* 1KMT was continuous during challenge test. The first mortality was observed at day 9 in G1, and day 15 in subgroup G2B. This disease resistance of *Oreochromis niloticus* against *Vibrio* parahaemolyticus 1T1 infection in subgroups G2A and G2B could be due to improvement of the fish immune system observed previously by *Lactobacillus plantarum* 1KMT. Indeed, according to Banerjee et al. [58], although the mechanism of activation of the innate and/or adaptive immunity by probiotics in

Table 4. Innate immunity parameters after 60 days feeding

Parameters	Treatment (60 days)	
	G1 (Control)	G2
Serum bactericidal activity (%)	75.50 ± 3.84^a	49.25 ± 2.86^b
Serum complement activity	25.40 ± 1.01^a	38.20 ± 1.32^b
Serum IgM level	705.62 ± 15.30^a	770.87 ± 21.69^b
Serum lysozyme activity (µg/mL)		
0 min	19.17 ± 0.23^a	24.37 ± 0.49^b
15 min	19.32 ± 0.19^a	24.65 ± 0.42^b
30 min	19.37 ± 0.28^a	24.7 ± 0.27^b
45 min	19.35 ± 0.40^a	24.85 ± 0.45^b
60 min	19.57 ± 0.37^a	25.02 ± 0.36^b

Values with different letters in superscript on the same line differ significantly ($p < 0.05$)

fish is not well described, the binding of the probiotic strain to the intestinal mucosa would have a stimulating effect on the innate and/or adaptive immune response, which allow the host to effectively resist to the infections [58]. These results suggest that dietary *Lactobacillus plantarum* 1KMT can exert positive effects on the health of cultured Nile tilapia.

Table 5. Challenge study during 21 days of trial

	Treatment		
	G1 (Control)	**G2A**	**G2B**
First mortality	Day 9	-	Day 15
Mortality (%)	77.7	0	66.6

G1: group fed with free-probiotic basal diet; G2A: subgroup in which treatment with probiotic was continue during challenge test; G2B: subgroup in which treatment with probiotic was stopped before challenge test

4. CONCLUSION

From the above results, probiotic *Lactobacillus plantarum* 1KMT strongly improve intestinal microflora, survival rate, growth performance and innate immunity parameters in *Oreochromis niloticus*. This strain can be used safely and efficiently in aquaculture to reduce some infections and improve productivity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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