

Effects of dietary probiotic supplementation, *Lactiplantibacillus plantarum* strain Be7 on growth and survival of *Oreochromis niloticus*

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Abstract

The aquaculture sector has experienced disruptions in its ability to feed the expanding population due to the existence of disease outbreaks in Nile tilapia (*Oreochromis niloticus*) cultivation. Antibiotic treatment in mitigating disease may not be the best alternative because it harms both humans and the environment. To solve this issue, probiotics as a feed supplement seems very promising as an alternative. This research aimed to study the effects of probiotic supplementation *Lactiplantibacillus plantarum* strain BE7 on the growth and survival of the *O. niloticus*. In this research, 2 groups were studied, which were the treatment and control groups. Juvenile *O. niloticus* in the treatment group was fed with fish feed coated with *L. plantarum* BE7 at a concentration of 1×10^9 CFU/mL (1 mL/g) while in the control group, the *O. niloticus* was fed with fish feed only. The feeding was administered with a feeding rate of 5% of the bodyweight twice daily at 09:30 and 15:30 for 35 days. Each group consists of three replicates. The growth performances observed in this research were survival rate, death rate, absolute growth rate of weight, length, and width, specific growth rate (SGR), and feed conversion ratio (FCR). Dissolved oxygen (DO), total dissolved solids (TDS), pH, and temperature were the water quality parameters measured. The results showed that the juvenile *O. niloticus* fed with probiotic had a better survival rate, and higher absolute growth weight, length and width, as well as, specific growth rate ($P > 0.05$). The treatment group also was recorded a lower death rate and feed conversion ratio compared to the juvenile fed without probiotics. The water quality was observed not affected by the presence of the probiotic in the water. The probiotic supplementation in the *O. niloticus* diet may help the digestive system of the fish by improving its feed digestibility; therefore, more nutrients are absorbed from fish feed, resulting in higher growth performance. As a conclusion, probiotic diet improves growth and survival of *O. niloticus*.

1. Introduction

The introduction of probiotics as a supplementary diet has been widely utilised in the food industry. The Greek word "biotic," which means "bios" or "life," and the Latin preposition "pro," which means "for," are the sources of the term "probiotic" (Ozen and Dinleyici, 2015). Lactic acid bacteria, or LAB, are probiotics that are usually used in the food production process in order to give benefits to the consumer. Bacteria in the lactic acid group include *Lactobacillus*, *Streptococcus*, and *Enterococcus* (Gopal, 2021). Probiotics are widely recognised to offer a variety of positive health impacts on both people and animals (Gopal, 2021). Few studies

have shown that the implementation of probiotic treatment into animal feed as a supplementary diet brings many benefits to the animal and its consumer.

According to the Food and Agriculture Organization (FAO), the world fish production by aquaculture had increased from 25.7% in 2000 to 46% in 2018 (Leong *et al.*, 2023). Fish production has increased due to the increasing number of fish consumed by the growing global population, which exceeds the consumption of all other animal proteins like meat, dairy and poultry (Leong *et al.*, 2023). Malaysia has built at least 49 freshwater and marine fish species aquaculture facilities

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(Department of Fisheries Malaysia, 2020). The most cultivated freshwater fish species were riverine catfish, tilapia, and catfish in 2020 (Department of Fisheries Malaysia, 2020). Nile tilapia (*Oreochromis niloticus*) has become more popular in recent years, surpassing other varieties of fish because of the rapid growth rate and its ability to survive in harsh environments, making it widely cultivated all over the world (Geletu and Zhao, 2022). However, fish farming does present certain difficulties, such as a slow growth rate that is caused by the presence of pathogens in the culture system, causing disease outbreaks.

The bacterial diseases that have been identified in the aquaculture industry include *Aeromonas* septicemia, Edwardsiellosis, Columnaris, Streptococcosis, and vibriosis (Irshath et al., 2023). Streptococcosis is a common fish disease caused by *Streptococcus agalactiae*, causing a large amount of mortality in the tilapia aquaculture (Ridzuan et al., 2022). In January 2020, a 70% mortality rate of red hybrid tilapia was reported due to the presence of Tilapia Lake Virus (TiLV), *Aeromonas hydrophila* and *S. agalactiae* outbreaks in a tilapia farm in Selangor (Basri et al., 2020). As the aquaculture industry tries to satisfy the rising demand for tilapia output during illness outbreaks, farmers employ an excessive amount of antibiotics that contribute to the development of antimicrobial resistance in the aquatic environment (Haenen et al., 2023). The presence of antimicrobial resistance in aquaculture endangers the aquatic environment and the health of the consumers. Therefore, probiotic treatment is highly recommended in aquaculture as a better alternative compared to antibiotic treatment because it is more environmentally friendly in maintaining sustainable aquaculture practices.

Probiotic treatment produces better fish products in terms of size and disease resistance during disease outbreaks. *Oreochromis niloticus* that were treated with *Lactiplantibacillus plantarum* strain L03 at a dietary supplementation of 5 g *L. plantarum* per kg showed improvements in feed utilisation, cellular immunity, and disease resistance (Nguyen et al., 2023). Probiotics from *Bacillus* could greatly boost the growth of healthy bacteria in the digestive tract of *O. niloticus*, which can lower the risk of illness and aid in better nutrition absorption and digestion (Jahangiri et al., 2018). In this study, *L. plantarum* strain BE7 was used to supplement the diet of *O. niloticus*. *Lactiplantibacillus plantarum* strain BE7 is a probiotic bacterium isolated from Malaysian fermented food, belacan (Ilyanie et al., 2023). This strain could tolerate acidic and bile environments of the human digestive tract, not exhibiting β -haemolysis on blood agar, demonstrating considerable antagonistic

activities against common food-borne pathogenic bacteria and displaying different resistance towards multiple antibiotics (Ilyanie et al., 2024). Despite its multiple probiotic potential, no actual research has been done to study the effect of this specific probiotic strain as feed supplementation on the growth of fish in aquaculture. Therefore, the objective of this research is to investigate the effect of probiotic treatment, *L. plantarum* BE7, on the growth performance and survivability of *O. niloticus*.

2. Materials and methods

2.1 Experimental design

The experiment was carried out at Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah. A total of ninety healthy juveniles *O. niloticus* with an average weight of 2.31 ± 0.39 g were obtained from the local fish farmer in Parit Tinggi, Kuala Pilah, Negeri Sembilan. The fish were divided into a control group and a treatment group. For each group, there were three replicates. The fish were placed into a transparent polypropylene 88-litre box ($68 \times 50 \times 39$ cm) that was filled with 78 mL of aerated dechlorinated water at 24°C. Each tank was set up with an air pump (RS Electrical Aquarium Air Pump Rs-248A) to allow more gaseous exchanges between the surrounding atmosphere and water. Before starting the experiment, ten *O. niloticus* were weighed using a digital weighing scale to obtain the initial weight. The length and width of the fish were also measured to obtain the initial measurement. The dissolved oxygen (DO) and total dissolved solids (TDS) were measured using a Digital Dissolved Oxygen Meter DO9100, while the pH and temperature were measured using the PH-686 Water Quality Tester. The water was changed 80% every 3 days, and the walls of each tank were scrubbed to remove the dirt before refilling with new aerated dechlorinated water.

2.2 Feed preparation

Pure culture of *L. plantarum* strain BE7 was stored in the chiller at 4°C. The probiotic was inoculated in De Man, Rogosa and Sharpe (MRS) broth and incubated overnight at 37°C. The next day, the overnight culture was centrifuged at maximum speed for 1 min, the supernatant was removed, and the pellet was resuspended in phosphate buffer solution. The overnight culture was diluted with phosphate buffer solution until the concentration of probiotics reached 1×10^9 mL⁻¹ (Wangyun et al., 2019). The probiotic count in the supplemented feed was 1×10^9 colony forming units (CFU) g⁻¹ feed.

2.3 Feeding management

The fish was fed twice per day at 9:30 a.m. and 3.30 p.m. (Cadorin *et al.*, 2022) for 35 days with a commercial diet (GOLD COIN 988 Min 30% protein, Max 6% fibre, Max 4%, Max 13% moisture). For the treatment group, the fish were fed with feed coated with the probiotic *L. plantarum* solution at the ratio of 1 mL/g. For the control group, the fish were fed with feed coated with phosphate saline buffer solution only at the same ratio. The feed was air-dried at room temperature for 1 hr to allow it to fully coat with the solution. The fish in both groups were fed at a rate of 5% of their body weight (Deyab and Hussein, 2015). Every time the feeding was performed, after 1 hr, the residual feed on the surface of the water in the tank was collected. The residual feed was dried out in order to determine the amount of dry weight of feed consumed by fish daily.

Feeding rate = $5\% \times (\text{Average body weight of fish}) \times \text{Total number of fish}$

2.4 Examination of growth performance

The growth parameters were measured according to:

$$\text{Survival rate (\%)} = N_t / N_0 \times 100$$

$$\text{Death rate (\%)} = (N_0 - N_t) / N_0 \times 100$$

Where N_t and N_0 are the final and initial number of the juvenile.

Absolute Growth of Weight/Length/Width = $(W_t/L_t/w_t - W_0/L_0/w_0) / t$

Where $W_t/L_t/w_t$ and $W_0/L_0/w_0$ are the final and initial weight/length/width of the juvenile, and t is the number of feeding periods.

$$\text{Specific growth rate (SGR)} = (\ln W_t - \ln W_0) \times 100 / t$$

Where W_t and W_0 are the final and initial weight of the juvenile, and t is the number of feeding periods.

Feed conversion ratio (FCR) = Dry weight of feed consumed (g)/ weight gain of fish (g).

2.5 Water quality analysis

The water quality for both groups of juveniles that were fed with probiotic treatment *L. plantarum* and without probiotic treatment was measured weekly. The water parameters that were measured include the dissolved oxygen, total dissolved solids, pH and temperature.

2.6 Statistical analysis

The mean comparison of the data between the treatment and control group ($P < 0.05$) was analysed using the Statistical Package for the Social Sciences (SPSS).

3. Results and discussion

3.1 Survival and death rate

The survival rate of juvenile *O. niloticus* in the treatment group (fed with fish feed coated with *L. plantarum*) was observed to be higher than the control group (Figure 1). Meanwhile, the mortality of the juvenile *O. niloticus* in the control group was slightly higher than the juvenile *O. niloticus* in the treatment group in the last week of the feeding trials (Figure 2). This could have happened because of the presence of the probiotic diet in the treatment group, which may give the juvenile a stronger immune system against pathogen infection that may cause mortality among fish compared to the fish in the control group (Raheem *et al.*, 2021). *Lactiplantibacillus plantarum* boosted innate immune indicators in *O. niloticus*, including lysozyme and peroxidase activity in skin mucus, alternative complement, phagocytosis, and respiratory burst activities (Doan *et al.*, 2018). The inclusion of probiotics in the diets of the treatment group increased the likelihood that the juvenile would survive compared to the control group by excluding the possible factors that lead to mortality among the juvenile (Hamka, 2020). The mortality in both groups may have occurred due to

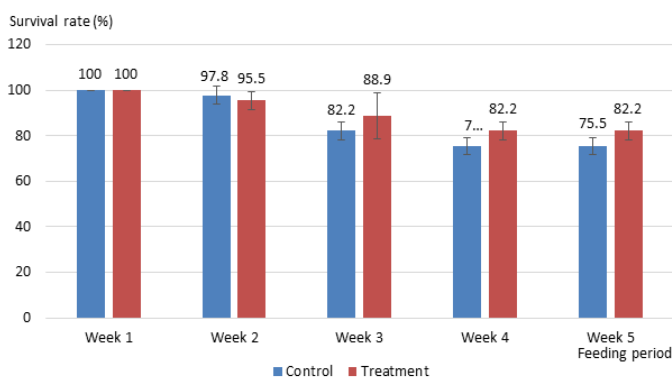


Figure 1. Survival rate of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

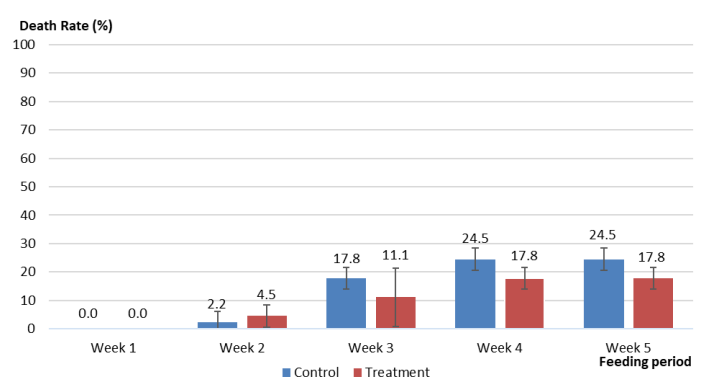


Figure 2. Death rate of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

external factors such as a limited food source, extra competition for space in the tank, and stress (Opiyo et al., 2020).

3.2 Absolute growth rate of weight, length, and width

The treatment group's absolute growth rate of weight, length, and width was increased slightly faster than the control group, although there was no significant difference between the two groups ($P>0.05$) (Table 1). This might have happened because the experimental period was too short for obtaining significant results between the two groups. Previous studies showed that the probiotic diets increased *O. niloticus* development and body composition (Opiyo et al., 2020). Based on several previous studies, the feeding trial period was recommended to be longer than 5 weeks in order to obtain higher absolute growth of weight, length, and width in the juvenile (Sutthi and Thaimuangphol, 2020). This is because a longer period of experimentation will allow the probiotic strain used to exert its beneficial effect on the *O. niloticus*, resulting in a better growth rate. The number of deaths in this study may also have hampered the significance of the comparison between the two groups.

Table 1. Absolute growth rate of weight, length, and width of *O. niloticus* after 35 days feeding trials.

Absolute growth of weight/length/width	Treatment group	Control group
Absolute growth of weight/day	0.13±0.01	0.1±0.01
Absolute growth of length/day	0.08±0.01	0.06±0.03
Absolute growth of width/day	0.03±0.01	0.03±0.01

3.3 Specific growth rate

The SGR in the treatment group (0.033 ± 0.001) was recorded as higher than the control group (0.026 ± 0.002). However, the SGR value for both groups did not show any significant difference ($P>0.05$) (Figure 3). The supplementation of *L. plantarum* BE7 with a concentration of 10^9 CFU/ mL in the feed diet caused the juveniles in the treatment group to grow slightly bigger compared to the control group. Previous research on the use of *L. plantarum* L-137 and/or β -glucan (BG) to treat Nile tilapia to increase their resistance to *A. hydrophila* revealed that the final body weight and SGR of the Nile tilapia significantly ($P<0.05$) increased values compared to the control group (Dawood et al., 2020). Another study showed that the data on the specific growth rate of tilapia under the treatment of adding probiotics grew higher than the control, according to observations conducted on tilapia rearing for 35 days (Sinaga and Mukti, 2022).

Despite the fact that there is a little growth difference between the two groups in SGR values in this study, the

inclusion of probiotics in the feed eventually led to an increase in fish production in the aquaculture sector. In treatments treated with 20% heat-killed *L. plantarum* L-137, compared to the control treatment, growth performance measures (average daily gain, specific growth rate, final total length, and length gain) show a considerably higher value at 90 days of the sampling period (Ekasari et al., 2023). Increased amount of feeding, concentration of probiotic and the period of feeding trials with *L. plantarum* supplementation for this study may have a strong likelihood of accelerating aquatic creature growth.

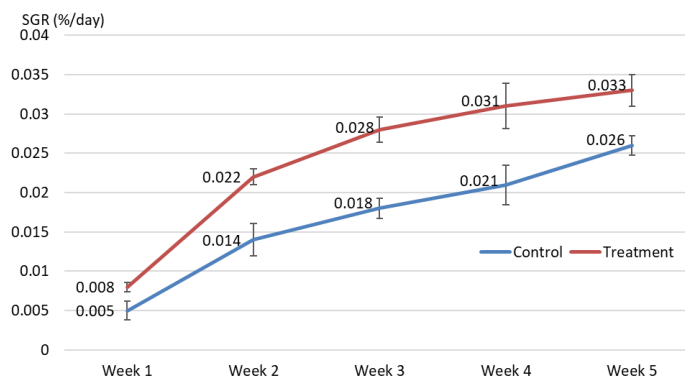


Figure 3. Specific growth rate of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

3.4 Feed conversion ratio

Feed conversion ratio (FCR) was measured to compare the feeding efficiency value of fish, which is converted into fish body weight gain, between juveniles that were fed with a probiotic diet (*L. plantarum*) and without probiotic during 35 days of feeding trials. In aquaculture, the weight of feed consumed divided by the weight of the animal is known as the feed conversion ratio (FCR), which is a common indicator of livestock production efficiency. The FCR value for the treatment group was observed to be lower than the control group (Figure 4). The FCR value for the treatment group is lower because the presence of probiotics in the feed may be able to improve the digestion ability of the juvenile *O. niloticus*, however, there is no significant difference recorded in both groups ($P>0.05$). Fish gut microbiota composition has been shown to be significantly impacted by probiotic feeding. This ultimately results in better fish growth by influencing the digestion and absorption of nutrients from the meal (Standen et al., 2015).

The presence of *L. plantarum* may increase the digestion process in juvenile *O. niloticus* in the treatment group by releasing digestive enzymes, including cellulase, amylase, and protease. Plus, probiotics also improve gut function, intestinal cellular integrity, inflammation control, pathogen inhibition, release of anti-virulence factors, defence against free radicals, and

improved immunity (Nathanailides *et al.*, 2021). Thus, these conditions help the juvenile to digest the feed efficiently. Therefore, less amount of feed is consumed by the juvenile to grow and develop (Hai, 2015). The lower the feed consumed by the juvenile, the lower the FCR value obtained in this study. This demonstrates that the juvenile that fed with probiotics may utilise the feed efficiently to support its growth and development, since the better for the fish, the smaller the conversion value of fish feed is.

experiment was recorded above 4 mg/L, which is good for the tilapia to grow (Riche and Garling, 2003) (Figure 5). For the total dissolved solids, every week it was recorded below 400 ppm (Boyd *et al.*, 2016), which is are recommended level for freshwater fish like *O. niloticus* to grow (Figure 6). The pH value for both groups was within the optimum range, which was 6.1 to 8.3 (Makori *et al.*, 2017) (Figure 7). Lastly, for the temperature, for both groups, it was recorded at the favourable temperature of Nile tilapia to grow, which is 20°C to 35°C (Lim *et al.*, 2006) (Figure 8). In short, the presence of the probiotic diet did not cause any changes in the temperature of the tank of *O. niloticus* during the experimental period. Therefore, the probiotic application as a supplementary diet is found not to cause any harm to the juvenile or the quality of the water. The usage of probiotic treatment can be concluded to be safe as a feed supplement to use in the aquaculture sector.

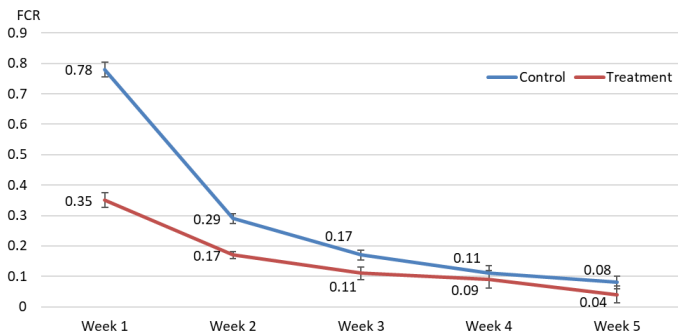


Figure 4. Feed conversion ratio of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

3.5 Water quality analysis

The water parameters in both groups that were fed with probiotic, *L. plantarum* and without probiotic were shown to be in a normal range for the juvenile tilapia to grow. The dissolved oxygen for each week of the

4. Conclusion

The supplementation of probiotic *L. plantarum* BE7 in fish feed for 35 days of feeding trials improves survival rate, absolute growth rate of weight, length and width, and specific growth rate (SGR). It also lowers the death rate and feed conversion ratio (FCR). *Oreochromis niloticus* did not exhibit any noticeable differences in

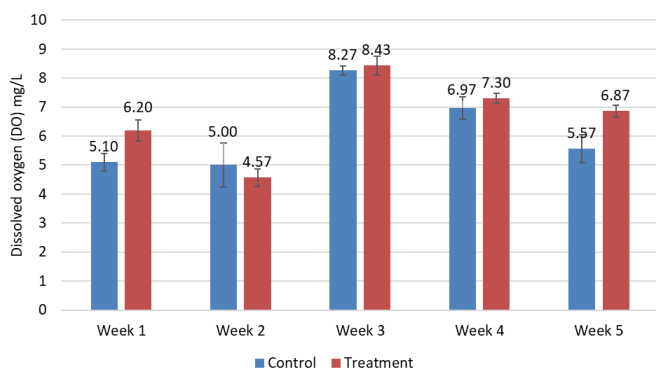


Figure 5. Dissolved oxygen of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

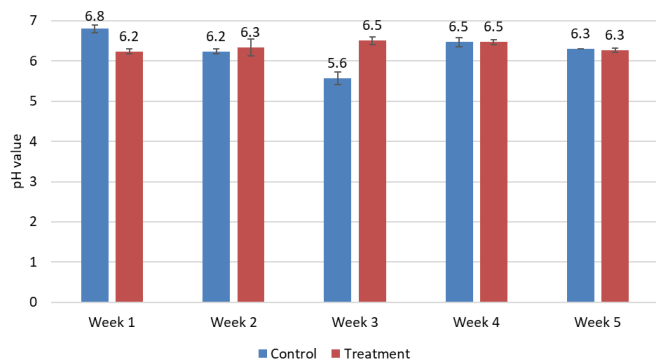


Figure 7. pH of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

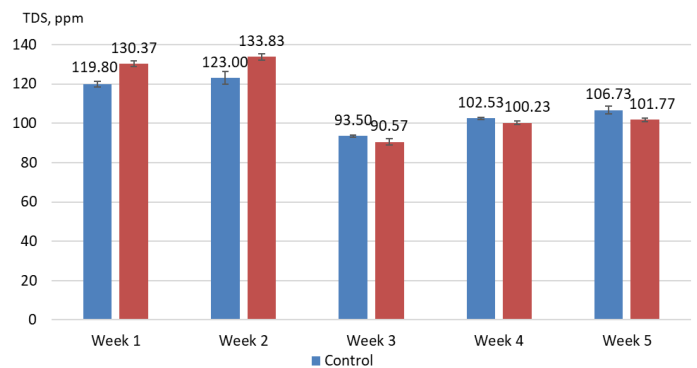


Figure 6. Total dissolved solids of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

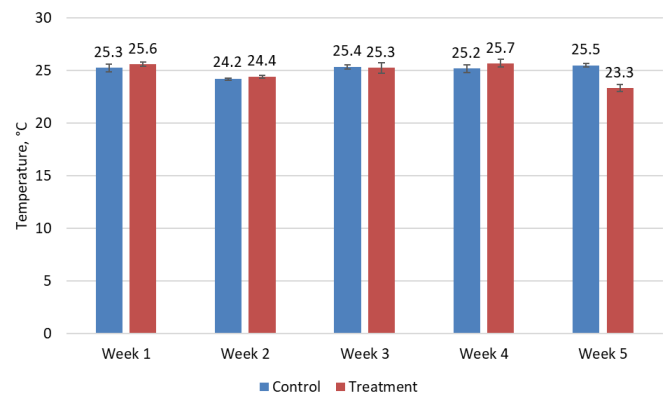


Figure 8. Temperature of *O. niloticus* during 35 days of feeding with probiotic diet (*L. plantarum*) and without probiotic diet (control).

growth or survival based on the concentration of probiotics *L. plantarum* BE7 10^9 CFU/mL added to their daily diets. Each tank's water quality in the treatment group was unaffected by the presence of probiotic supplementation *L. plantarum* BE7. The presence of probiotic (*L. plantarum*) does not cause any changes in achieving an optimum level of water parameter when compared to the juveniles of *O. niloticus* that were fed without probiotic. More research is required to determine the effectiveness of this probiotic strain as a treatment in the aquaculture system. The use of probiotics *L. plantarum* in growing juvenile *O. niloticus* is strongly advised in order to promote growth and survival by employing larger dosages and a longer trial time in order to gain more significant differences between both groups.

Conflict of interest

The authors declare no conflict of interest.

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