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Dietary Probiotics as a Modulator of Hematological Parameters in *Maylandia greshakei*

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Abstract

Probiotics have emerged as promising alternatives to antibiotics for improving fish health and productivity in sustainable aquaculture. The present investigation evaluated the influence of dietary *Lactobacillus acidophilus* supplementation on the growth performance and hematological profile of the ornamental cichlid *Maylandia greshakei*. Fish were cultured for 60 days and fed experimental diets containing 0 (control), 2, 4, and 6 mg of *L. acidophilus* per 100 g of feed. Growth indices, including weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate, were assessed, while hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT) were determined after 30 and 60 days of feeding. Dietary probiotic supplementation significantly enhanced growth performance, with the highest probiotic level (6 mg/100 g feed) producing superior weight gain (11.92 ± 0.15 g), SGR (3.71 ± 0.03), feed utilization efficiency, and a survival rate of 97%. Fish receiving probiotic diets also exhibited improved hematological status, reflected by elevated RBC, WBC, Hb, and Hct values, suggesting enhanced oxygen-carrying capacity and immune response, whereas MCV, MCH, MCHC, and PLT showed no significant variation among treatments. These findings indicate that dietary *Lactobacillus acidophilus*, particularly at 6 mg/100 g feed, effectively promotes growth, improves hematological health, and enhances survival in *Maylandia greshakei*, highlighting its potential as a safe and sustainable feed additive for ornamental fish aquaculture.

Keywords: *Maylandia greshakei*, *Lactobacillus acidophilus,* probiotics, hematology, growth performance, ornamental fish and sustainable aquaculture.

Introduction

Aquaculture practices involved using high-quality feeds with high crude protein content which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and result in good growth performance. Aquaculture industry is faced with the challenges of inadequate supply and high cost of quality fish feeds. Beside the problem of high cost of fish feeds and quality seed, disease outbreak is a major challenge in fish farming.

Antibiotics have typically been used to prevent and control disease in intensive fish farming. However, studies have reported an increase in resistance to pathogens and growth in aquaculture systems (Verschuere *et al*., 2000). The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. Probiotics are usually defined as live microbial feed supplements, that are administered in such a way as to enter the gastrointestinal tract and to be kept alive; this beneficially affects the host animal by improving its intestinal microbial balance and in turn its health (Gatesoupe, 1999 and Gomez *et al.,* 2000). The use of probiotics in human and animal nutrition is well documented and recently, they have begun to be applied in aquaculture.

Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Verschuere *et al*., 2000).

The present study aimed to determine the effects of dietary supplementation with commercial probiotic *Lactobacillus acidophilus* on growth performance and blood hematological parameters of ice blue cichlid, *Maylandia greshakei* with an experimental period of 60 days.

Materials and Methods

Experimental fish

The experimental fish *M. greshakei* (Ice blue cichlid) were purchased from Saravanan fish farm, Sawyerpuram at Thoothukudi district and transported to the laboratory. The fishes were fed with control diet, which was prepared by using the following ingredients like fish meal, *anchovy, jawala* meal, soy flour, tapioca flour and wheat flour for a period of 15 days. After ensuring complete evacuation of gut by starving them for about one day, the fishes of uniform weight were selected (length: 8±0.3 cm, weight: 16±0.5gm) using digital electronic balance. Thirty fishes of *M. greshakei* were introduced into each cement tank (3m x 1.5m x1m) and triplicates were maintained for each experimental diet. The bacteria *L. acidophilus* were obtained from the Madurai Kamaraj University, Madurai, Tamil Nadu, India. The experimental diets were fed thrice a day (9am, 1pm and 5pm) at a rate of 5% of its body weight per day. Water quality parameters like pH, temperature, dissolved oxygen, total alkalinity and ammonia were measured periodically following standard methods (APHA, 1998). Every four days, ammonium, nitrite and nitrate concentrations were checked and if the values exceeded the critical levels, the water was exchanged (but not more than 10% daily) in order to maintain the values within the optimal range. In the present study, four experimental diets were prepared by incorporating the probiotic *L. acidophilus* in the concentrations of *La1* (2mg), *La2* (6mg) and *La3* (6mg) (Table 1).

Table 1: Experimental diets and dose of the present study

Type of Diets	Probiotic Bacteria	Dose
Control	Standard diet + Without probiotics	---
*La*1	Standard diet + *L. acidophilus*	2mg/100g feed
*La*2	Standard diet + *L. acidophilus*	4mg/100g feed
*La*3	Standard diet + *L. acidophilus*	6mg/100g feed

Growth performance indices

The experimental fishes were weighed after the experiment was over and the following growth performance indicators were calculated: Weight Gain (WG), Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR). The following formulae that were used to find out the above said indices.

$$\text{Weight Gain (WG)} = \frac{\text{Final weight (Wt)}}{\text{Initial weight (W0) (g)}}$$

$$\text{Food Conversion ratio (FCR)} = \frac{\text{Total feed (F)}}{\text{Total weight gain (W) (g/g)}}$$

$$\text{Specific Growth Rate (SGR)} = \frac{(\ln Wt - \ln W0)}{t\ (\%\ BW/day)} \times 100$$

Blood sampling

After the completion of the experimental duration (60 days), 0.5 ml of blood samples was drawn by caudal vessels with the lithium heparin coated needle and syringe. Among the experimental samples, five fishes from each group were randomly selected for the hematological study. The hematological study includes Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb), Haematocrit (Hct), Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelets Count (PLT).

Red Blood Cells Count (RBC)

The red blood cell counts (RBCcx10^6/µl) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian dilute solution.

Hematocrit (Hct) Level

The hematocrit (PVC,%) was determined, in duplicate, by using heparinised capillary tubes and centrifuged for 5 minutes at 12000 rpm in a micro hematocrit centrifuge.

Haemoglobin (Hb) concentration

Haemoglobin concentration (Hb, g/dl) was determined by photometrical cyanmethaemoglobin method.

Red Blood Indices

Red blood indices were calculated by using the standard formula (Ghergariu *et al.,*1985).

The Mean Corpuscular Volume (MCV, µm3) was calculated by using the following formula:

$$\text{MCV} = \frac{\text{Hct}}{\text{RBC}}$$

The Mean Corpuscular Haemoglobin (MCH, pg) was calculated by using the following formula:

$$\text{MCH} = \frac{\text{Hb}}{\text{RBC}}$$

The Mean Corpuscular Haemoglobin Concentration (MCHC, g/dl) was calculated by using the following formula:

$$\text{MCHC} = \frac{\text{Hb}}{\text{Hct}}$$

The growth performances and haematological parameters of the different experimental groups were expressed by mean and standard deviation (M±SD) and the differences between the values were statistic analyzed with t-Student test.

Results

Growth Performance

After the experimental period of 60 days, no mortality was detected in all the experimental groups and there was a significant difference between the mean weights of different experimental groups from *La*1 to *La*3 and control. The highest weight gain (g) was noticed as 11.92±0.15 g in *La*3 although the mean weight in each group increased with increase in concentration of probiotics. The mean weight of *M. greshakei* of each treatment group was significantly higher than that of the control (9.96 ± 0.02). The highest SGR values were obtained in *La*3 (3.71±0.03) followed by *La*2 (11.10±0.22) and *La*1 (10.11±0.01) and *La*0 (9.96±0.02). The results obtained for WG and SGR in all groups treated with probiotics at all concentrations were significantly higher ($P<0.05$) than those of the control. The highest FCR (1.60±0.09) was observed in *La*3 followed by *La*2 (1.58±0.12), *La*1 (1.49±0.15) and *La*0 (1.34±0.11). The highest survival rate was observed in *La*3 (97%) followed by *La*2 (94%), *La*1 (92%) and control (89%). These results confirmed that the probiotics at higher concentration produced elevated values in growth parameters of *M. greshakeii* (Table 2).

Table 2: Growth performance of *M. greshakei* fed with experimental diets.

Parameters	Control	*La*1	*La*2	*La*3
Initial number of fish	30	30	30	30
Final number of fish	30	30	30	30
Initial weight (g)	16.10 ± 0.04^{a}	16.55 ± 0.02^{a}	16.60 ± 0.04^{a}	16.70 ± 0.03^{a}
Final weight (g)	26.06 ± 0.41^{b}	26.66 ± 0.01^{b}	27.70 ± 0.08^{b}	28.62 ± 0.13^{c}
WG (g)	9.96 ± 0.02^{b}	10.11 ± 0.01^{b}	11.10 ± 0.22^{a}	11.92 ± 0.15^{a}
SGR	3.26 ± 0.01^{b}	3.47 ± 0.01^{a}	3.62 ± 0.01^{a}	3.71 ± 0.03^{b}
FCR	1.34 ± 0.11^{b}	1.49 ± 0.15^{a}	1.58 ± 0.12^{a}	1.60 ± 0.09^{b}
Survival rate (%)	89%	92%	94%	97%

The mean values having different superscripts in the same row are significantly different at $p<0.05$ % level and ± indicates the standard deviation

Table 3: Length increment of *M. greshakei* during the experimental period

Diets	Mean initial Length (cm)	Length at the day of 60		Length increment after 60 days	
		Male	Female	Male	Female
Control	8.5±0.3	10.8	11.0	2.3	2.5
*La*1	8.5±0.3	11.1	11.2	2.6	2.7
*La*2	8.5±0.3	11.3	11.6	2.8	3.1
*La*3	8.5±0.3	11.5	11.9	3.0	3.4

The table 3 shows that, the length increase of *M. greshakei* during the experimental period of 60 days. The higher length increment was observed in *La*3 (3.0 cm in male and 3.4 cm in female) followed by *La*2 (2.8 cm in male and 3.1 cm in female), *La*1 (2.6 cm in male and 2.7 cm in female) and control (2.3 cm in male and 2.5 cm in female).

Blood hematological parameters

Table 4: Effects of different levels of probiotics on hematological parameters in 30 days of the experiment

Blood Parameters	Control	*La*1	*La*2	*La*3
WBC (103µL^{-1})	161.4±11.05^{a}	181.4±32.12^{a}	202.5±6.44^{a}	210.6±5.13^{a}
RBC (106µL^{-1})	2.17±0.23^{a}	2.47±0.22^{b}	2.68±0.20^{ab}	2.88±9.12^{ab}
Hb (gdL^{-1})	6.78±1.22^{a}	7.61±1.21^{a}	8.65±0.43^{a}	9.54±0.31a
Hct	22.90±2.55^{b}	24.63±2.35^{b}	25.83±2.25^{b}	26.71±2.15^{b}
MCV(fl)	121.41±3.24^{a}	119.7±3.19^{a}	112.9±1.79^{a}	126.3±3.12 ^{a}
MCH(pg)	45.9±4.54^{a}	43.6±10.1^{a}	40.0±1.67^{a}	49.7±0.52^{a}
MCHC(gdL^{-1})	39.7±1.61^{a}	37.5±2.15^{a}	36.6±1.24^{a}	38.9±2.15^{a}
PLT (103µL^{-1})	124.22±49.11^{a}	77.83±20.36^{a}	109.3±11.47^{a}	95.3±34.28^{ab}

The values are represented as Mean±SE, Means with the same letter for each parameter are not significantly different at p>0.05

The table 4 shows that the hematological characteristics of *M. greshakei* fed with different levels of probiotics. The blood sample was collected at 30th day showed that the WBCs of all fish fed varying inclusion of probiotic was not significantly different (p>0.05), while Fish fed control diet (0% probiotic) has the lowest RBCs count and was not significantly different from fish fed with *La*1 and *La*2 (2mg, 4mg of probiotics) but significantly different from fish fed with *La*3 (6mg of probiotic). There was no significantly difference among (p>0.05) the Hb, MCV, MCHC, MCH and PLT of fish fed all experimental diet.

During the experiment, blood samples were collected on 60th day shows that the white blood cell count of fish fed the control diet was significantly different (P<0.05) from fish fed with *La*3 (6mg) but was not significantly different (P>0.05) from WBC of fish fed with diet *La*1 and *La*2 (P>0.05). There was no significant difference in WBC of fish fed diet *La*1, *La*2 and *La*3. The red blood cell count (RBC) shows no significant difference (P>0.05) in the blood parameter of fish fed *La*0, *La*1 and *La*3 but there was significant difference (P<0.05) in the red blood cell of fish fed with and control diet. There were no significant differences (P>0.05) in Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Platelets count (PLT) of the blood of all fish fed with varying concentration of probiotic (Table 5).

Table 5: Effects of different levels of probiotics on hematological parameters in 60 days of the experiment

Blood Parameters	Control	*La*1	*La*2	*La*3
WBC (103µL¯1)	168.2±11.62[a]	181.4±32.12[a]	202.5±6.14[a]	208.2±4.63[a]
RBC (106µL¯1)	2.02±0.17[a]	2.47±0.61[b]	2.68±0.10[ab]	2.86±9.82[ab]
Hb (gdL¯1)	7.68±1.41[a]	7.62±1.01[a]	8.67±0.45[a]	9.93±0.14a
Hct (%)	21.09±2.12[b]	23.32±2.35[b]	24.93±1.97[b]	26.71±1.91[b]
MCV(fl)	121.41±2.81[a]	118.92±3.01[a]	113.12±1.73[a]	129.43±2.87[a]
MCH(pg)	44.72±4.13[a]	43.9±9.78[a]	40.4±1.61[a]	47.21±0.35[a]
MCHC(gdL¯1)	41.12±0.78[a]	38.11±2.01[a]	35.23±1.42[a]	42.12±2.02[a]
PLT (103µL¯1)	121.3±53.33[a]	77.33±20.02[a]	110.3±12.31[a]	95.15±33.91[ab]

The values are represented as Mean±SE, Means with the same letter for each parameter are not significantly different at p>0.05

Discussion

All different concentrations of probiotic diet resulted in improved growth performances and food conversion ratio was also better in these diets than that of the control diet, suggesting that addition of probiotics could reduce the culture cost of *M. greshakei*. Hidalgo *et al*., (2006) observed that *Bacillus cereus* administered in the host *Dentex dentex* (2g/kg) showed better FCR and SGR. Similar results were recorded in *La3* in *M. greshakei*. Balcazar *et al.*, (2007) reported that *B. subtilis* UTM 126 strain showed better growth in *Litopenaeus vannamei*. In their study four potential probiotic strains were taken and followed by immersion challenge against *A. hydrophila* exhibited resistance. Among the four *B. subtilis* showed better results when compared with the control. *B. subtilis* AB1 was able to effectively protect rainbow trout against *Aeromonas sp*. (Cunningham- Randles and Lin, 1998). There are ample references to prove *Bacillus* sp is beneficial in protecting against bacterial pathogens. The probiotic bacteria like *Vibrio* sp. *B. subtilis* showed a higher antibiosis and also used to improve pond water quality, leading to the enhanced survival of black tiger prawns (Vaseeharan *et al*., 2004). The *Lactobacillus* incorporated diet can be used as a fish fed in *Clarias gariepinus* induce the fish health, better survival, feed efficiency and growth performance (Ayoola *et al.,* 2013). According to Newaj – Fyzul *et al*., (2009) *B. subtilis* AB1 stimulated both the humoral and cellular immune responses, which provided the rainbow trout with adequate protection to survive the challenge by the highly vigorous *Aeromonas* sp. *B. subtilis* AB1 influenced the production of higher level of lyzozyme activity in fish serum and gut mucus (Panigrahi *et al*., 2005). In the present study, *La*3 showed the best specific growth rate (3.71±0.03), weight gain (11.92±0.15) and the best FCR (1.60±0.09). The same trend was observed by Gatlin *et al*., (2007). They also reported that *B. subtilis* can be used effectively as a commercial product for use in aquaculture.

Hematological parameters have been widely used to diagnosis of disease and pathologies of human and domestic animals. The hematological techniques are one of the important tools for fishery biologists in assessing the fish health and to monitoring stress responses. Some of the recorded values were fluctuating due to the condition under which the fishes were kept, the condition based on the fact that the fishes are not in their natural habitat and also because of sizes of the fishes, blood values such as the white blood cell, red blood cell and haemoglobin value. This was reported by Osuigwe *et al*., 2005, that the hematological parameters of fish are affected by a range of factor which includes size, age, physiological status, environmental conditions and dietary.

The hematology results of the present study showed that WBC, RBC, PCV and PLT were affected by diet treatment. At 30 and 60 day blood of fish fed diet with 0.5g of probiotic have the highest WBC count. The RBC was higher in fish fed the probiotic diet than control diet at 30 and 60 day. The obtained result has merged with the result of Marzouk *et al.,* (2008). He found increases in RBCs count, HB value, PCV and WBCs count in fish groups fed with diet supplemented with probiotic (*B. subtillis* and *Saccharomyces cerevisae*). From this result we came to know that the probiotic can be considered as an alternative method to antibiotics for feed supplement and health improvement in aquaculture at low inclusion level.

Conclusion

The result of the sixty days feeding trial of *Maylandia grashakei* with probiotic *Lactobacillus acidophilus* showed that probiotic could be used in fish production. However growth performance and blood parameter (especially white blood cell and red blood cell) could be improved in fish by incorporating probiotic at 4mg/100g feed. It can be deduce from this research that feed incorporated with mixture of *Lactobacillus acidophilus* can be used as a fish feed in ice blue cichlid *Maylandia grashakei* to enhance fish health, survival, better feed efficiency and growth performance. However, I recommend more research more work on this probiotic under various conditions and probiotic should be made commercially available for aquaculture use at reasonable price.

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