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Research Article

Does commercial probiotics improve the growth performance and hematological parameters of Nile tilapia, *Oreochromis niloticus*?

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ABSTRACT

Oreochromis niloticus becoming a promising aquaculture species globally, but recent disease outbreaks and poor growth with commercial feed making it challenging. A 60 days long aquarium trial and series of laboratory assays have been conducted to assess the growth performance of *O. niloticus* fed with a locally available commercial probiotic. *O. niloticus* fry's were fed with a mixture of basal diet and probiotics supplementation at a level of 0% (control), 0.2%, 0.4% and 0.8%. After the trial phase weight gain, length gain, specific growth rate (SGR), percentage of weight gain (PWG), percentage of length gain (PLG) were noted. Among all, highest values of above parameters were observed at T_1 (0.2%) treatment group. Weight gain, length gain, PLG and PWG were significantly improved in T_1 treatment group ($p<0.05$). Additionally, hematological parameters including hemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC) were also observed for all groups and T_1 was found to have highest values for all these parameters, although there were no statistically significant differences between the values of T_1 and T_2. The results of this study showed that 0.2% dietary probiotics supplements in basal diet would optimize the growth performance and hematological parameters of aquarium reared *O. niloticus*.

Keywords: Probiotics, Growth performance, Hematological parameters, *Oreochromis niloticus*

Introduction

A native fish group of Africa continent, tilapias are among the most practiced species in aquaculture industry worldwide as well as in Bangladesh (Alam et al., 2014; Akter et al., 2019) due to their high productivity rate, disease tolerance and flesh quality (Yuan et al., 2017; Gabriel, 2019;). The commercial hatcheries in Bangladesh produced all male mono-sex fry to adopt rapidly growth rate as well as to reduce undesirable reproduction in culture pond (Lind et al., 2015; Das et al., 2019). Use of chemicals, hormones, drugs and probiotics are getting very popular among aquaculture practices in Bangladesh (Uddin et al., 2017). The need for high-quality fish feeds with a premium protein content, associated nutrients and minerals; which is tasty, keeps animals healthy and providing a high growth rate is increasing (Soltan et al., 2016; Hua et al., 2019; Yue et al., 2020). Probiotics are combination of live microorganisms that are efficient to adapt, colonize and grow within the gut of the host and develop a beneficial stability of microorganisms to improve animals health (Martínez Cruz et al., 2012; Carbone & Faggio, 2016). Numerous benefits of probiotics for growth, defense and intestinal health of the host were revealed and broad use of probiotics in aquaculture could prevent diseases, promote growth and reduce the extensive use of antibiotic (Austin & Austin, 2016). Probiotics retard or completely inhibit the growth of pathogenic bacteria following a competitive exclusion (Akayli et al., 2016), also boost up the immune response and secretion of mucosal enzymes to promote host growth and they do not cause secondary pollution problems (Xia et al., 2020). Variations in fish blood parameters would be a good pointer of water quality, nutrition and health (Satheeshkumar et al., 2012; Ahmed et al., 2020). Alterations in hematological parameters are due to the result of stress condition such as hypoxia, contact to pollutants, transportation, handling and liberation of energy associated with the use of chemicals and anesthetics (Roche & Bogé, 1996; Fazio et al., 2015; Simide et al., 2016). Therefore, the present research was directed towards the evaluating the growth of *O. niloticus* fed with dietary probiotics as well as determining the optimum supplementation level to produce an effective diet, which would provide a favorable physiological condition to culture this species commercially in Bangladesh.

Material and Methods

Experiment Designing and Diet Preparation

A 60 days long trial have been conducted in 140 litre glass aquaria. The experiment was designed with four treatments designated with three replications as well. A commercial floating fish feed (moisture 11%, protein 40 %, lipids 6%, carbohydrate 25 %, 450 fiber 5%, ash 10%, calcium 2% and phosphorous 1 %) was used. A commercial probiotics mixture (AquaStar growout powder; Renata animal health Ltd. Bangladesh) that contains *Bacillus, Enterococcus, Pedicoccus* and *Lactobacillus sp.* bacteria was added in diets of experiments groups with a rate of 0% (Tc), 0.2% (T1), 0.4% (T2) and 0.8% (T3). Tilapia (*O. niloticus*) fry's were acclimated to laboratory conditions for 14 days and fed only with commercial feed. *Then* twenty fish with a mean weight of 1.72 $\pm$0.42g were randomly allotted into each aquarium. They were fed three times a day at 6% of their body weight at the first month of experiment and gradually reduced to 5% in the second month. Underground freshwater which was stored in a reservoir and supplied to the aquaria. Each aquarium was equipped with automated aeration and internal carbon filtration facilities. Uneaten feed and the waste materials of aquarium were siphoned out twice per day and approximately 20 percent water was exchanged every two days to keep the water environment suitable for fish survival.

Monitoring Water Quality Parameters and Fish Sampling

Various water quality parameters such as temperature (with a simple thermometer), dissolved oxygen (YSI digital DO meter, model 58) and pH (pH meter - Hanna Instruments, Japan) in each aquarium was monitored once in a week during the experiment period. Every 15 days, three fishes from each aquarium were sampled randomly in all treatments groups for length and weight gain after a 24 hours starvation period.

Analysis of Growth Parameters

At the end of the feeding trial various growth parameters were analyzed by using the mathematical formula according to Olvera-Novoa et al., (1990), Panase & Mengumphan, (2015) and Pechsiri & Yakupitiyage, (2005).

Weight gain= Mean value of final weight- Mean value of initial weight

Percentage of weight gain

$$= \frac{\text{Mean value of final fish weight} - \text{Mean value of initial fish weight}}{\text{Mean value of initial fish weight}} \times 100$$

Specific growth rate SGR (%) $= \frac{ln W2 - ln W1}{T2 - T1} \times 100$, Where W_1= the initial body weight (gm) at a time, W_2= the final body weight (gm) at a time, T_2-T_1= Duration in days

Length gain=Mean value of final length- Mean value of initial length

Percentages of length gain

$$= \frac{\text{Mean value of final fish length} - \text{Mean value of initial fish length}}{\text{Mean avlue of initial fish length}} \times 100$$

Average daily weight gain

$$= \frac{\text{Mean value of final weight} - \text{Mean value of initial weight}}{\text{Duration of experiment in days}}$$

Average daily length gain

$$= \frac{\text{Mean value of final length} - \text{Mean value of initial length}}{\text{Duration of experiment in days}}$$

The values of Fulton's condition factor (K) was estimated by plotting length weight data on the following equation adopted from Htun-Han, (1978); $K=(W/L^3)*100$

Blood Sample Collection and Hematological Analysis

Blood samples were collected (3 fishes from each group) after a 24 hours starvation period from the caudal vena and stored in EDTA (Ethylene diamine tetra-acetic acid). Hemoglobin, WBC, and RBC analysis was carried out by using Automated Hematology Analyzer BC-3000 Plus.

Data Analysis

The one-way analysis of variance (ANOVA) and Duncan's multiple Range Test (DMRT) were conducted to figure out the differences among the groups means at significance level of P<0.05. All statistics were carried out using Statistical Package for Social Science (IBM SPSS) version 22.

Results and Discussion

In this study tilapia fry were feed with a standard commercial feed and with the addition of various amounts of a probiotic mixture and the differences in growth and blood parameters in fish were revealed.

Water quality parameters are vital as they influence the growth and physiological activities of fish (Maucieri et al., 2019). Temperature is a key factor for the production management and feed consumption in fish. The optimal thermal range for the proper growth of *O. niloticus* was proposed as 25-27 °C (Makori et al., 2017) and 27-32 °C (Mengistu et al., 2020). Dissolved oxygen, which is a crucial factor for fish growth, health, and physiology should be over 5 mg/L for sustainable growth of *O. niloticus* (Riche & Garling, 2003; Makori et al., 2017). pH is an imperative factor which specifies the health and production output of a water body and optimum range was proposed as 5.5-9.0 (Rebouças et al., 2016) and 6.1-8.3 (Makori et al., 2017) for *O. niloticus*. Water quality parameters i.e., temperature, dissolved oxygen (DO) and pH observed during the study were shown in Table 1. These results showed that the water quality parameters were appropriate for *O. niloticus* culture.

Among four experimental groups fed with basal commercial feed and probiotic mixture - 0% (Tc), 0.2% (T1), 0.4% (T2) and), 0.8% (T3) - maximum mean weight gain was detected in T_1 (16.1975 ±3.16g) followed by T_2 (12.79 ±3.16g) and T_3 (10.326 ±2.47g) respectively (Table 2). The lowest mean weight was observed in T_C (8.23 ±1.83g) and the means of the weight gains among all the treatments groups were significantly varied between each other (P<0.05). Among the groups, T_1 (0.2%) showed the highest weight gain and T_C (0%, Probiotic) showed the lowest growth performance. The mean percentages of weight gain (PWG) in *O. niloticus* was recorded in T_C (478.86 ±204.86[a]), T_1 (981.52 ±382.27), T_2 (863.31 ±339.98) and T_3 (702.09 ±298.95) (Table 2). Highest mean PWG was found in T_1 followed by T_2, T_3 and T_C, respectively. However, difference between T_2 and T_3 (P>0.05) were statistically uniform and the lowest mean PWG was observed in control treatment. Specific Growth Rate (SGR%) of *O. niloticus* was recorded as 2.83 ±0.55, 3.87 ±0.57, 3.68 ±0.56 and 3.36 ±0.62 in T_C, T_1, T_2 and T_3 groups respectively (Table 2). Highest SGR value (3.87 ±0.57) was observed in T_1 while the lower SGR value was recorded in T_C (2.83 ±0.55) group. The differences between T_1, T_2, T_2 and T_3 diet groups (P>0.05) remained still statistically non-significant.

The mean length gain of *O. niloticus* was recorded as 4.38 ±0.84 cm, 8.02 ±1.09cm, 5.93 ±0.94 cm and 5.18 ±1.03 cm in T_C, T_1, T_2 and T_3 groups respectively (Table 2). The length gain was increased in T_1 groups followed by T_2, T_3 and T_C groups, respectively. The highest mean length was observed in T_1 diet group whereas the control group (T_C) showed the lowest mean length gain during 60 days of experiment. The difference among all groups were significant at P<0.05. The highest percentages of length (PLG) were observed as 184.44±48.27 in T_1 groups followed by T_2, T_3 and T_C groups, respectively. PLG (%) values 141.33 ± 36.84 and 120.04±33.94 were recorded in T_2 and T_3 groups respectively (Table 2). T_C (102.37±25.38) group showed the lowest percentage of length gain. T_1 group showed a significant difference than the other treatment but there is no significant difference between T_2 and T_3 groups (P>0.05) in terms of percentage length gain.

Table 1. Mean value of water quality parameters (Mean Value ±SD)

Water quality parameter	Experiment groups			
	T_C (commercial feed only)	T_1 (0.2% probiotics)	T_2 (0.4% probiotics)	T_3 (0.8% probiotics)
Temperature (˚C)	26.33±1.03[a]	26.67±1.03[a]	26.67±0.81[a]	26.5±1.04[a]
Dissolved oxygen (mg/L)	5.33±0.21[a]	5.55±0.08[a]	5.35±0.16[a]	5.41±0.12[a]
pH	7.43±0.08[a]	7.6±0.30[a]	7.5±0.28[a]	7.4±0.08[a]

Table 2. Growth parameters of *O. niloticus* after 60 days treatment (means ± standard deviation) (P>0.05)

Parameters	Experiment groups			
	T_C (commercial feed only)	T_1 (0.2% probiotics)	T_2 (0.4% probiotics)	T_3 (0.8% probiotics)
Mean Initial Weight (g)	1.90±0.56[a]	1.79±0.45[a]	1.59±0.42[a]	1.59±0.36[a]
Mean Initial Length (cm)	4.36±0.49[a]	4.46±0.50[a]	4.30±0.49[a]	4.42±0.44[a]
Mean Final Weight (g)	10.13±1.92[a]	17.99±3.01 [d]	14.38±3.11[c]	11.92±2.31[b]
Mean Final Length (cm)	8.75±0.81[a]	12.48±0.83[d]	10.23±0.73[c]	9.60±0.76[b]
Weight Gain (g)	8.23±1.83[a]	16.19±3.16[d]	12.79±3.16[c]	10.32±2.47[b]
Length Gain (cm)	4.38±0.84[a]	8.02±1.09[d]	5.93±0.94[c]	5.18±1.03[b]
% Weight Gain	478.86±204.86[a]	981.52±382.27[c]	863.31±339.98[b]	702.09±298.95[b]
% Length Gain	102.34±25.38[a]	184.44±48.27[c]	141.33±36.84[b]	120.04±33.94[a, b]
SGR %	2.83±0.55[a]	3.87±0.57[c]	3.68±0.56[b, c]	3.36±0.62[b]
ADWG	0.13±0.03[a]	0.26±0.05[b]	0.21±0.05[c]	0.17±0.04[d]
ADLG	0.07±0.014[a]	0.13±0.018[b]	0.09±0.015[c]	0.086±0.017[d]
Condition factor, K	1.78±0.23[a]	1.19±0.22[b]	1.39±0.19[b, c]	1.55±0.31[d]

SGR= Specific growth rate, **ADWG**= Average daily weight gain, **ADLG**= Average daily length gain.

Supplementation of probiotics in the diet of aquatic animal increased enzymatic activity, developed digestive activity, synthesis of vitamins and weight gain which enhance the growth of fish (Reyes-Becerril et al., 2008; Nayak, 2010) and modulate immune response (Giri et al., 2013; Galagarza et al., 2018). The dietary supplementation of probiotic and bacterial cocktails were found to improve the gut immune response, morphology and microbial assemblage of intestine in juvenile *Oreochromis niloticus* (Ayyat et al., 2014; Yamashita et al., 2017; Xia et al., 2020). In this study, supplementation of probiotics in all experiment groups resulted higher growth than the control group (Table 2). It might be occurred due to proper digestion and better nutrient absorption in the fish body. The optimum probiotic level that resulted high in terms of weight gain (g), length gain (cm), SGR (%), Percentage of weight gain, percentage of length gain growth of *O. niloticus* was found in T_1 (0.2% probiotic) diet group. This indicated that the overall better growth performance was found in T_1 group. Similar observations have been reported on *Labeo rohita* (Munirasu & Ramasubramanian, 2017), *Clarias gariepinus* (Al-Dohail et al., 2009) and *Catla catla* (Bandyopadhyay & Das Mohapatra, 2009). All the above study had proven that growth performance of these fishes was meaningly improved in the diet containing probiotic containing than those in control.

Lower SGR (%) was observed in T_C group (2.83 ±0.12g) but among the probiotics treatments T_3 (3.36 ±0.13) showed the lowest SGR (%) rate (Table 2). However, there was no significant improvement among the treatment groups in case of SGR (%). However, there is possibility of arising different toxic elements along with the secretion of enzyme which may hinder the growth or other parameters of fish (Rahman et al., 2019; Chen et al., 2020) and while using very high dosage of probiotics and better growth performance might not be always associated with higher concentration of the probiotic (Ghosh et al., 2008; Mahmoud et al., 2021). A previous study on same species reported highest weight gain at 0.2% probiotics dietary supplement group in compared with the control groups (Chowdhury et al., 2020).

The condition factor (K) represent the nature of physical factors and biological regulating the growth of fish and it is found to be influenced by a set of factors including feeding types and stress associated with parasitic and physiological agents (Hartman & Margraf, 2006; Datta et al., 2013; Shoko et al., 2015; Jisr et al., 2018). The k>1 indicate a healthy environment of animals surroundings (Golam Mortuza & Al-Misned, 2013; Asmamaw et al., 2019;). The value of k has been reported above 1 and significantly varied between different treatment groups (Table 2), which indicate the quality of water, feed, and animal welfare on current research.

Hematological parameters represent a better illustration about fish health and environmental monitoring (Eissa & AbouElGheit, 2014; Dowidar et al., 2018) and they are influenced by various factors including animal's size, growth phase, physiological position, diet and overall environmental circumstances (Cho et al., 2015; Parrino et al., 2018). Highest mean hemoglobin (Hb) value was recorded in T_1 (5.70 ±0.17 g/dL) compared to T_2 (5.30 ±0.30 g/dL), T_3 (4.56 ±0.20 g/dL) and T_C (3.76 ±0.25g/dL) respectively (Table 3). Insignificant differences of Hb was observed between T_1 and T_2 groups (P>0.05). Control group showed a lower level of Hemoglobin. In case of mean white blood cell (WBC) counts, there were also no significant different between T_1 and T_2 (P>0.05). The highest WBC was observed in T_1 (10.89 ± 0.55 × 10^4/cumm) followed by T_2 (10.15 ± 0.64 ×10^4/cumm) (Table 3). The mean amount of red blood cell (RBC) was higher in T_1 (1.19 ±0.06 m/µL) compared to the other groups (P>0.05) (Table 3). The T_C groups showed significantly lower level of RBC.

The present research has been revealed that dietary probiotics supplementation increases hemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC) contents in all the groups compared with the control group (Table 3). The fish fed with probiotic mixed food became more nutritious due to declined cortisol levels in the plasma haemolymph (Carnevali et al., 2006; Rollo et al., 2006; Al-Dohail et al., 2009) and high cortisol level increase glucose in blood which seems an indicator of physiological stress in fish (Silva et al., 2015). The high level of hemoglobin in fish fed with probiotic might be occurred due to the increasing of iron absorption in blood mediated through releasing acids in gut (Mohapatra et al., 2014; Silva et al., 2015). Firouzbakhsh et al., (2011) stated that a rise in the number of RBC increases the overall hemoglobin concentration in fish blood. In WBC Count T_1 (0.2%, probiotic) and T_2 (0.4%, Probiotic) were insignificantly higher than the other treatments and this blood contents are engaged in modulation of innate immunity via phagocytosis and toxic cell formation (Chico et al., 2018; Puente-Marin et al., 2019). These indicate that the strong immune system might positively affect the health and growth of fish.

Table 3. Blood parameters of *O. niloticus* in different groups (means ± standard deviation) (P>0.05)

Parameters	T_C (commercial feed only)	T_1 (0.2% probiotics)	T_2 (0.4% probiotics)	T_3 (0.8% probiotics)
Hb (g/dL)	3.76 ± 0.25^a	5.70 ± 0.17^c	5.30 ± 0.30^c	4.56 ± 0.20^b
WBC (x10^4/cumm)	5.58 ± 1.16^a	10.89 ± 0.55^c	10.15 ± 0.64^c	7.64 ± 2.42^b
RBC (m/µL)	0.70 ± 0.133^a	1.19 ± 0.064^c	$0.99\pm0.056^{b,c}$	0.76 ± 0.18^b

***WBC**= White blood Cell, **RBC**= Red Blood Cell, **g/dL**= gram/deciliter, **cumm**= cubemeter, **m/µL**= million/microliter.

Conclusion

The present research was conducted for the determination of the optimum probiotics level in feed to obtain to obtain a better growth of *O. niloticus.* The results of this study showed that probiotic had a higher impact on the growth performance and some blood parameters of *O. niloticus.* After considering the overall performance, it can be concluded that 0.2% dietary probiotics can be the optimum to provide a better growth performance of *O. niloticus*. The addition of this dietary level of this probiotic mixture may be used in commercial culture of this species. In addition, further study should be designed to observe the result of probiotics in addition to other additives on the cultured growth of tilapia as well as other species.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Approved by institutional, regional and national animal ethical statements.

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