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Research Article

Effects of Probiotics, Prebiotics, and Synbiotics as an Alternative to Antibiotics on Growth and Blood Profile of Nile Tilapia (*Oreochromis niloticus*)

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The purpose of this study is to compare the effects of probiotics, prebiotics, and their synergism against antibiotics on the growth performance and hematology of Nile tilapia (*Oreochromis niloticus*). The study was designed by using five treatments over 75 days in which T_{Con} (control), T_{Ant} (antibiotics: Cotrim vet), T_{Pro} (commercial probiotics: pond care), T_{Pre} (prebiotics: spirulina), and T_{Syn} (synbiotic: probiotic and prebiotic) were used. All the treatments showed significant ($P < 0.05$) improvements in growth and feed utilization parameters. The highest mean final growth was found in T_{Syn} (13.79 ± 0.11) and then in T_{Pre} (13.61 ± 0.02), T_{Pro} (13.22 ± 0.12), T_{Ant} (10.04 ± 0.7), and T_{Con} (8.89 ± 0.19). Weight gain and specific growth rate were significantly ($P < 0.05$) different between treatments. The food conversion ratio varied significantly ($P < 0.05$) across treatments and the lowest value was observed in T_{Pro} (1.13 ± 0.021). The protein efficiency ratio (PER) was significantly ($P < 0.05$) different between treatments and the highest value was in T_{Pro} (3.1 ± 0.05). The survival rate was not significantly ($P < 0.05$) different between treatments. The highest white blood cell (WBC) counts were found in T_{Syn} (12.63 ± 0.11) followed by T_{Pre} (12.6 ± 0.2), T_{Pro} (12.32 ± 0.12), T_{Con} (12.16 ± 0.105), and T_{Ant} (11.03 ± 0.46). The values of red blood cells and platelets were not statistically significant ($P < 0.05$) among the different treatments. However, all treatments showed normal hepatocyte structure in liver tissue histology, and intense hepatic lipid vacuoles in T_{Syn} indicated improved growth. The higher growth performance and feed utilization values were observed in probiotics, prebiotics, and their synergism units compared to antibiotic treatment. Therefore, probiotics, prebiotics, and their synergism can be used as an alternative to antibiotics in tilapia aquaculture.

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the economically important farmed fish cultured extensively on the global perimeter [1, 2]. Along with a growing population, the demand for food is increasing, and also the demand for cultured species is increasing significantly [3]. The overall global production of tilapia attained nearly 6 million in 2020 [4]. Tilapia is considered one of the most promising fish for meeting up food security in Asian and African countries [5, 6]. Tilapia is the third most cultured single species in Bangladesh contributing 9.95% of annual inland fisheries production while per capita consumption of fish is 62.58 g/day [7]. Tilapia has high market demand as a low-cost protein source among consumers while protein content varied from 14.93% to 16.03% in tilapia collected from different sources in Bangladesh and, it is also rich in unsaturated fats, vitamins, minerals, and trace elements [8]. Due to its low cost, high nutritional value also having no health hazards tilapia is preferred among consumers [9]. As aquaculture practices have become more intensified, stressful conditions are more prevalent in aquatic animals and are also responsible for the deterioration of the surrounding environment [10, 11]. These stress conditions are

leading to infectious diseases, and to control them, certain chemical compounds and antibiotics are more frequently used in aquaculture. Extensive antibiotic use has become more popular to avoid any kind of disease risk, which can cause mass mortality and a huge loss to farmers. Most antibiotics are widely used without having any dose limitations or regulations, which causes a great risk to human health. Fish provided antibiotics for a long time become resistant to antibiotics as they retain the strains of bacteria. Different formulations of sulfamethoxazole and trimethoprim (TMP) are one of the extensively used antibiotics groups in aquaculture [12, 13]. Aquaculture discharges have been marked as one of the major sources of antibiotic pollution in the environment [14]. An antibiotic has a residual effect and can be transmitted to humans through the consumption of fish, making the consumer resistant to bacteria and increasing antibiotic resistance.

The alternative to antibiotics is concerned that they will be cost-effective, reduce disease risk, and not have any residual or harmful effects. Different substances are being tested, and probiotics, prebiotics, and synbiotics are still being considered the most favorable. A probiotic is a feed supplement containing mostly live microbial organisms that benefit the host animal [15]. It improves the host's intestinal microbial balance, controls microbiota, and feed efficiency, confers disease resistance, and improves water quality [10, 16]. Different gram-positive and gram-negative bacteria have been used as probiotics in aquaculture, which has provided positive benefits. *Bacillus* spp. is widely used as a probiotic in aquaculture due to its ability to survive in the fish gut and secretes enzymes that help in digestion thus improving the host's specific growth rate and reducing food conversion ratio and also producing different antimicrobial substances [17]. *Bacillus*-based probiotics improved digestion enzyme activities, growth, and the intestinal microbiota of tilapia [18]. *Bacillus amyloliquefaciens* improved Nile tilapia survival, growth, disease resistance, and immune response [19]. *Bacillus cereus* boosts the immune response of Nile tilapia [18]. *Aspergillus oryzae* is a filamentous fungus and is used as a probiotic enhanced growth performance and blood profile in Nile tilapia [20]. Prebiotics are fibers derived from plants or sugar conversed food materials that are indigestible to the host but can be utilized by gut bacteria, and their use increases beneficial gut commensal bacteria [21, 22]. Prebiotics contributes to stimulating selected favorable indigenous microbial populations, resulting in improvements in the host's health. By competing for the same glycoconjugates on the surface of epithelial cells, prebiotics can inhibit pathogenic microorganism's adhesion and invasion selectively in the colon epithelium, preserving the nonspecific barrier function and improving the production of mucus, short-chain fatty acids, and cytokines [23]. Different prebiotic substances such as inulin, beta-glucans, mannan oligosaccharide, spirulina, and different yeasts have proved to be beneficial for fish immune systems while providing extra benefit in enhancing growth [24–26]. Spirulina (*Arthrospira platensis*), a type of blue-green algae, has been known to improve the gut microflora of aquatic animals when used at an optimum level [27, 28] and improve water quality by reducing nitrogenous substances [29]. Spirulina has high crude protein content and is rich in vitamins, minerals, and different bioactive compounds, and supplementation in lower amounts in fish feed can act as a growth promoter [30]. Spirulina supplementation in fish diets improves the fish growth [31, 32]. Spirulina used in aquaponic systems showed enhanced growth in Nile tilapia [33]. Synbiotics are supplements where probiotics and prebiotics in the form of synergism provide nutritional benefits [34]. Different synbiotics supplements improved fish growth and health status [35, 36]. All these are used as feed supplements in aquaculture to improve disease resistance and enhance growth and survivability in fish and shellfish. Antibiotics have a harmful effect on ecology, and both sulfamethoxazole and trimethoprim are broadly used in fish culture in our country. A cost-effective and environmentally friendly alternative to these antibiotics is required for reducing animal health risks. To that end, the effect of probiotics, prebiotics, and their synergism on Nile tilapia is being evaluated in a current study.

2. Materials and Methods

2.1. Animals Ethics. The experiment design and study species have been approved by the animal ethics committee of Sylhet Agricultural University Research System and the Department of Fish Biology and Genetics. This research had been duly approved by all sorts of institutional, regional, and national animal ethics conditions.

2.2. Study Location and Experimental Design. The study was carried out at the "Biofloc Lab and Mini Hatchery" of the Department of Fish Biology and Genetics, Sylhet Agricultural University, Bangladesh for a period of 75 days from February 22 to May 7, 2021. This experiment was conducted at a total of 15 aquariums, comprising five treatments each having three replications. Each aquarium had 103993.31 cm^3 (73.5 cm × 36.75 cm × 38.5 cm) volume, and fishes were reared in 80 L water. The water exchange rate was 10% and groundwater was used. The five treatments were T_{Con}, T_{Ant}, T_{Pro}, T_{Pre}, and T_{Syn}. The treatments T_{Con} reflects for control, T_{Ant} for antibiotic (Cotrim vet), T_{Pro} for probiotic (Pond Care), T_{Pre} for prebiotic (Spirulina), and T_{Syn} for synbiotic (probiotic and prebiotic).

2.3. Experimental Diet. T_{Con} was designated as a control where only commercial diet was treated. A commercially formulated pellet diet, with brand name Mega Feed, Spectra Hexa Feed Ltd., Bangladesh was used as a basal diet. Chowdhury et al. [37] discovered that Mega Feed has a crude protein content of 27.94%, a fat content of 2.81%, a fiber content of 6.45%, an ash content of 9.49%, and a moisture content of 8.21%. This proximate composition analysis was confirmed by the Quality Control Laboratory, Department of Fisheries, Savar, Bangladesh. T_{Ant} was administering the antibiotic Cotrim Vet (Square Pharmaceuticals Ltd, Bangladesh) at a level of 0.015 $g.kg^{-1}$ body weight of fish

according to the manufacturer's recommended dose limit into 80 L water daily. Cotrim Vet has a chemical constitution of sulfamethoxazole BP 500 mg and trimethoprim BP 100 mg in each 5 ml. T_{Pro} was the addition of 0.0035 g of commercial probiotic (Pond Care, SKF Biofloc Fish Probiotic, Bangladesh) into 80 L water daily. The dosage limit was followed by the company instruction, and the composition of commercial probiotic pond care is shown in Table 1. T_{Pre} was the mixing of Osaka Green 1 (Perfect Companion Ltd, Thailand) into commercial feed. Osaka Green 1 contains the prebiotic spirulina (*Arthrospira platensis*) and has a crude protein content of 28%, a crude fat content of 3%, a crude fiber content of 4%, and a 10% moisture content. About 5% of Osaka Green 1 was added to commercials following the previous description by Jana et al. [38]. T_{Syn} was a combination of both treatments T_{Pro} and T_{Pre}. Fish were fed the commercial diet at 4% of their body weight three times per day based on the previous efficient feeding strategies from An and Anh [39] and Limbu et al. [40].

2.4. Collection and Acclimatization of Tilapia Fry. Fry of Nile tilapia was collected from a private hatchery in Mymensingh, Bangladesh. The collected fry was acclimatized for 15 days before being stocked in the aquarium. A completely randomized design (CRD) was followed for equal and random distribution of tilapia fry. A total of 600 fish were used in this study while 120 fish were distributed in each treatment. Each treatment had 3 replications and 40 fishes were randomly distributed in each replication with an average weight of 0.53 ± 0.029 g.

2.5. Analysis of Growth and Feed Utilization. Sampling was done fortnightly to gather the weight data of tilapia. For accurate data, all fish from each tank were sampled. Weight data were taken using a digital balance. After 75 days, fish were collected from each aquarium and weighed and counted to determine growth and survival. Water quality parameters such as temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), ammonia, and salinity were recorded weekly from each aquarium between 9:00 am and 10:00 am. Temperature and DO were measured using a standard thermometer and dissolved oxygen test kit. pH, TDS, ammonia, and salinity were measured by using a pH meter, TDS meter, ammonia test kit, and refractometer, respectively. The following formulas were used to compute several growth metrics such as weight gain, percent weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), and survival rate [41, 42]:

Table 1: Composition of bacteria in commercial probiotic (pond care).

Bacteria	Concentration (CFU.g^{-1})
Bacillus subtilis	2×10^9
Bacillus licheniformis	2×10^9
Bacillus polymyxa	2×10^9
Bacillus pumillus	2×10^9
Bacillus megaterium	2×10^9
Bacillus coagulans	2×10^9
Bacillus amyloliquefaciens	2×10^9
Aspergillus niger	0.2×10^9
Aspergillus oryzae	1.2×10^9

$$
\begin{aligned}
\text{Weight gained} &= W_F - W_O,\\
\%\ \text{of weight gain} &= \frac{\text{Weight gained}}{W_O} \times 100,\\
\text{SGR}\left(\%.\text{da y}^{-1}\right) &= \frac{\{\text{Ln}\,(W_F) - \text{Ln}\,(W_O)\} \times 100}{\text{Cultured period (days)}},\\
\text{FCR} &= \frac{\text{Total amount of dry feed consumed}\ (g)}{\text{Wet weight gain of fish}\ (g)},\\
\text{PER} &= \frac{\text{Wet weight gain}\ (g)}{\text{Protein consumed}\ (g)},\\
\text{Survival rate} &= \frac{N_F}{N_I} \times 100.
\end{aligned}
\tag{1}
$$

Here, W_O is the initial body weight in grams, W_F is the final body weight in grams, and N_I and N_F are the initial number of stocked fish and the final number of survived fish, respectively.

2.6. Hematological Research. After completion of the experimental trial, fish were collected from each replication of each treatment for blood analysis. The standard procedure of blood collection has been followed as per the description of Hasan et al. [43] Fish were collected randomly using a scoop net and anesthetized with chloroform (Merck, Germany) to retain blood. The dose of the anesthetics was adjusted by prior repetitive trials as described by Carreiro et al. [44]. The process involved the evaporation of 0.5 ml chloroform in a specimen jar for every 12 fish. Placing the fish in a dissection tray, blood was collected from the caudal vein by syringe, and the collected blood was placed in a tube containing heparin anticoagulant to avoid coagulation. Approximately, 300–350 microliters of blood have been collected from each individual and three fishes have been

collected for each replication in the treatment group. The collected sample was analyzed on a digital hemocytometer. Blood glucose concentrations were measured by a commercial glucose test kit Clever Chek TD-4226 (Germany). The parameters measured were white blood cells, red blood cells, hemoglobin, and glucose.

2.7. Histological Examination. After taking all the parameters and data, the fish liver sample was taken for histological analysis. Histological analysis was done by following the methods of Hassaan et al. [45]. Three fish from each treatment unit were randomly chosen, euthanized, and dissected abdominally. The samples were fixed for 24 hours in neutral buffered formalin solution, dehydrated in ascending concentrations of ethanol, and cleaned with xylene. The sample was fixed in paraffin and later sectioned, with each section having a 4-5 μm thickness for histological investigations. Sections were stained with hematoxylin and eosin for general morphometry. A prepared slide was observed under an electron microscope, and digital images were taken by the attached digital camera, Carl Zeiss Primo Star 3.0 Microscope, Germany.

2.8. Economic Analysis. The total expense of feed, treatment, and other fixed costs was calculated during the study. The selling price was calculated based on per-inch size fingerling cost. The currency converted used as US$1 = BDT 84.81 [46]. The cost benefits for economic analyses were performed by following the description of Meah and Akther [47].

2.9. Statistical Evaluations. One-way analysis of variance (ANOVA) was used for statistical analysis. Duncan's multiple range tests, with a significance rate of $P < 0.05$, was utilized to evaluate variations in treatment meanings. The Statistical Package for Social Science (SPSS) version 26 and Microsoft Excel 2010 were used to conduct all statistical analyses.

3. Results

3.1. Environmental Factors. Table 2 shows the mean (±SD) values of the environmental factors measured at 7-day intervals during the experimental period. Water quality measures such as temperature, dissolved oxygen, pH, ammonia, and salinity were not significantly ($P < 0.05$) different in any of the treatments. In the water quality measure of total dissolved solids, there was a significant ($P < 0.05$) difference between treatments.

3.2. Growth Parameters. All the treatments had a significant ($P < 0.05$) effect on the growth of Nile tilapia compared to the control. Growth performance and feed utilization parameters of Nile tilapia including mean initial weight, mean final weight, weight gained, % weight gain, specific growth rate (SGR), feed conversion ratio (FCR), the protein efficiency ratio (PER), and survival rate, presented as means ± SD at different treatments after 75 days feeding period are shown in Table 3. There was no significant ($P < 0.05$) difference in mean initial weight (g) among all five treatments, but there was a significant ($P < 0.05$) difference in the mean final weight. The highest mean final growth was observed in T_{Syn} followed by T_{Pre}, T_{Pro}, T_{Ant}, and T_{Con}. The highest weight gain was found in T_{Syn} which was insignificant ($P < 0.05$) to T_{Pre} and T_{Pro} but significant ($P < 0.05$) to T_{Ant} and T_{Con}. The linear regression between treatments (x) and weight gain (y) was $y = 1.3534x + 7.3178$, with an $R^2 = 0.8617$ (Figure 1). The percentage weight gain was highest in T_{Syn} which was insignificant ($P < 0.05$) compared to T_{Pre} and T_{Pro} but significant ($P < 0.05$) compared to T_{Ant} and T_{Con} where $y = 317.18x + 1205.1$, $R^2 = 0.8807$, linear regression between treatments (x), and % weight gain (y) (Figure 1). T_{Syn} had the highest specific growth rate, which was insignificant ($P < 0.05$) compared to T_{Pre} and T_{Pro}, but significant ($P < 0.05$) compared to T_{Ant} and T_{Con}. Linear regression between treatments (x) and specific growth rate (y) was $y = 0.1993x + 3.5239$, $R^2 = 0.8711$ (Figure 1).

The food conversion ratio is an indication of feed efficiency, and lower values indicate higher feed efficiency. T_{Pro} had the lowest food conversion ratio, followed by T_{Pre} and T_{Syn}, then T_{Ant} and T_{Con}. There was a significant ($P < 0.05$) difference between treatments. Linear regression between treatments (x) and food conversion ratio (y) was $y = -0.0768x + 1.6004$, $R^2 = 0.5636$ (Figure 1). The highest protein efficiency ratio was in T_{Pro} which is significant ($P < 0.05$) compared to all other treatments. T_{Pre} is insignificant ($P < 0.05$) in comparison to T_{Syn} but significant ($P < 0.05$) in comparison to T_{Ant} and T_{Con}. The regression between treatments (x) and protein efficiency ratio (y) was $y = 0.155x + 2.148$, $R^2 = 0.5949$ (Figure 1).. The survival rate was not affected by treatments and the linear regression between treatments (x) and survival rate (y) was $y = 0.5x + 92.667$, with an $R^2 = 0.9$ (Figure 1).

3.3. Hematological Analysis. A detailed hematological analysis is given in Table 4 and values are presented as mean ± SD. The white blood cell value was highest in T_{Syn} which was not significant ($P < 0.05$) to T_{Pre} but significant ($P < 0.05$) to T_{Ant} and less significant ($P < 0.05$) to T_{Pro} and T_{Con}. The highest lymphocyte value was observed in T_{Con} and the lowest in T_{Pre}. The monocyte value was higher in T_{Pre} and the lowest was in T_{Con}. The highest granulocyte value was in T_{Pre} and there is no significant ($P < 0.05$) difference in values of lymphocyte, monocyte, and granulocyte between treatments.

The value of red blood cells was not significantly ($P < 0.05$) different in all five treatments. Red blood cells were highest in T_{Pre} followed by T_{Pro}, T_{Con}, T_{Ant}, and T_{Syn}. Hemoglobin value was highest in T_{Pro}. Mean corpuscular volume was highest in T_{Con} and lowest in T_{Syn}. Hematocrit and red blood cell distribution width were highest in T_{Pre}. Mean corpuscular hemoglobin was highest in T_{Ant} and mean corpuscular hemoglobin concentration was highest in T_{Syn}.The platelet value of all five treatments was not significantly ($P < 0.05$) different from each other. The platelets count was highest in T_{Syn} then in T_{Pre} followed by T_{Pro}, T_{Ant},

Table 2: Water quality parameters of the experimental tank during the study period.

Water quality	Treatment				
	T_{Con}	T_{Ant}	T_{Pro}	T_{Pre}	T_{Syn}
Temperature (°C)	22.23 ± 1.89^{a}	22.23 ± 1.89^{a}	22.23 ± 1.89^{a}	22.23 ± 1.89^{a}	22.23 ± 1.89^{a}
Dissolved oxygen (mg.L^{-1})	5.48 ± 0.13^{a}	5.51 ± 0.1^{a}	5.45 ± 0.09^{a}	5.51 ± 0.05^{a}	5.54 ± 0.09^{a}
p^{H}	7.226 ± 0.002^{a}	7.232 ± 0.003^{a}	7.16 ± 0.0^{a}	7.212 ± 0.002^{a}	7.178 ± 0.003^{a}
Total dissolved solids	41.67 ± 0.58^{c}	41.88 ± 0.68^{c}	50.76 ± 0.58^{b}	42.06 ± 0.52^{c}	55.33 ± 1.15^{a}
Ammonia	0.003 ± 0.000^{a}	0.004 ± 0.0^{a}	0.002 ± 0.0^{a}	0.004 ± 0.0^{a}	0.003 ± 0.000^{a}
Salinity (ppt)	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}

Table 3: Growth performance and feed utilization data of Nile tilapia (*Oreochromis niloticus*).

Parameter	Treatments				
	T_{Con}	T_{Ant}	T_{Pro}	T_{Pre}	T_{Syn}
Initial weight (g)	0.57 ± 0.014^{a}	0.56 ± 0.014^{a}	0.53 ± 0.025^{a}	0.51 ± 0.014^{a}	0.51 ± 0.014^{a}
Final weight (g)	8.89 ± 0.19^{b}	10.04 ± 0.7^{b}	13.22 ± 0.12^{a}	13.61 ± 0.02^{a}	13.79 ± 0.11^{a}
Weight gain (g)	8.33 ± 0.18^{b}	9.48 ± 0.69^{b}	12.7 ± 0.13^{a}	13.11 ± 0.02^{a}	13.28 ± 0.11^{a}
% weight gain	1470.12 ± 45.34^{b}	1696.46 ± 82.6^{b}	2422.54 ± 134.68^{a}	2579.62 ± 76.37^{a}	2614.45 ± 94.07^{a}
SGR (%.day^{-1})	3.67 ± 0.04^{b}	3.85 ± 0.06^{b}	4.3 ± 0.07^{a}	4.38 ± 0.04^{a}	4.4 ± 0.05^{a}
FCR	1.55 ± 0.037^{a}	1.34 ± 0.031^{b}	1.13 ± 0.021^{c}	1.22 ± 0.01^{c}	1.226 ± 0.018^{c}
PER	2.3 ± 0.05^{d}	2.6 ± 0.06^{c}	3.1 ± 0.05^{a}	2.93 ± 0.02^{b}	2.91 ± 0.04^{b}
Survival rate (%)	93.3 ± 1.44^{a}	93.3 ± 1.44^{a}	94.16 ± 1.44^{a}	95 ± 0.00^{a}	95 ± 0.00^{a}

Results were presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($P < 0.05$). T_{Con} = Control, T_{Ant} = antibiotic, T_{Pro} = probiotic, T_{Pre} = prebiotic, T_{Syn} = synbiotic (probiotic + prebiotic). SGR = Specific growth rate (%.day^{-1}), FCR = food conversion ratio and PER = protein efficiency ratio.

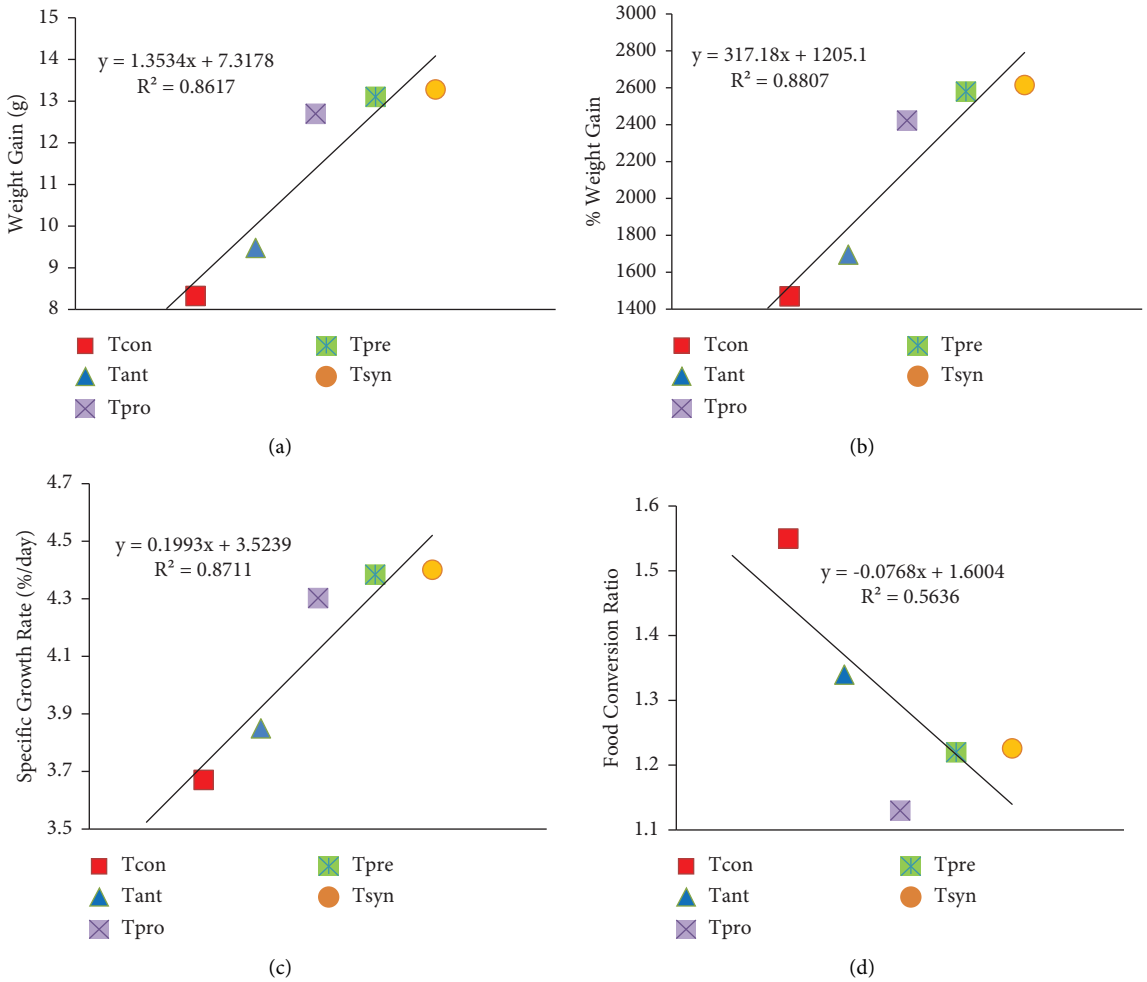

Figure 1: Continued.

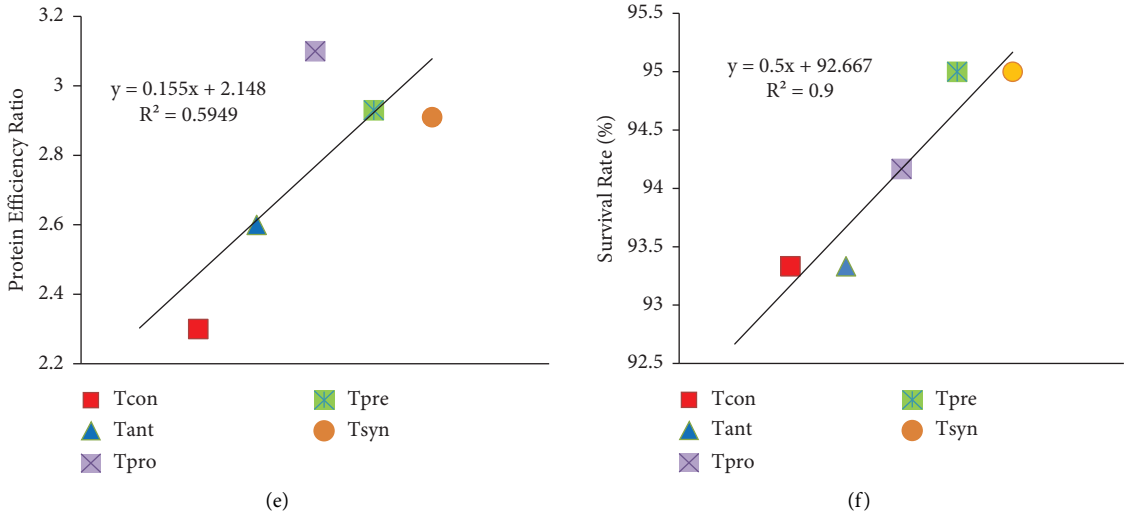

FIGURE 1: Linear regression between treatments and growth parameters. R^2 = Co-efficient of determination.

TABLE 4: Blood parameters of Nile tilapia (*Oreochromis niloticus*) after 75 days of experimental trial.

Parameters	Treatment				
	T_{Con}	T_{Ant}	T_{Pro}	T_{Pre}	T_{Syn}
WBC ($\times 10^4/m^3$)	12.16 ± 0.105^{ab}	11.03 ± 0.46^{b}	12.32 ± 0.12^{ab}	12.6 ± 0.2^{a}	12.63 ± 0.11^{a}
LYMP (%)	84.15 ± 0.95^{a}	79.4 ± 6.9^{a}	81.3 ± 1.2^{a}	77 ± 2.3^{a}	82.15 ± 1.45^{a}
MONO (%)	6.95 ± 0.25^{a}	7.9 ± 1.6^{a}	7.8 ± 0.4^{a}	8.7 ± 0.3^{a}	7.6 ± 0.3^{a}
GRA (%)	8.9 ± 0.7^{a}	12.7 ± 5.3^{a}	10.9 ± 0.8^{a}	14.3 ± 2.0^{a}	10.25 ± 1.15^{a}
RBC (million cell.μl^{-1})	2.24 ± 0.07^{a}	2.14 ± 0.015^{a}	2.31 ± 0.07^{a}	2.41 ± 0.14^{a}	2.13 ± 0.15^{a}
HGB ($g.dl^{-1}$)	8.45 ± 0.35^{a}	8.23 ± 0.15^{a}	8.63 ± 0.65^{a}	8.6 ± 0.40^{a}	7.53 ± 0.35^{a}
HCT (%)	39.4 ± 3.2^{a}	37.4 ± 1.25^{a}	36.73 ± 1.65^{a}	41.4 ± 1.41^{a}	31.23 ± 1.0^{a}
MCV (fL)	176.7 ± 18.23^{a}	174.53 ± 7.05^{a}	159.26 ± 11.95^{a}	172.18 ± 4.04^{a}	147.33 ± 14.82^{a}
MCH (pg/Cell)	37.87 ± 2.43^{a}	38.4 ± 0.9^{a}	37.33 ± 1.7^{a}	35.74 ± 0.35^{a}	35.4 ± 0.9^{a}
MCHC ($g.dl^{-1}$)	21.49 ± 0.86^{a}	21.99 ± 0.34^{a}	23.56 ± 2.85^{a}	20.73 ± 0.30^{a}	24.13 ± 1.88^{a}
RDW (fL)	23.4 ± 1.1^{a}	17.63 ± 2.67^{a}	16.76 ± 2.70^{a}	30.26 ± 13.30^{a}	27.36 ± 4.90^{a}
Platelet ($\times 10^4/m^3$)	8.78 ± 2.85^{a}	11.27 ± 1.19^{a}	12.77 ± 8.25^{a}	13.4 ± 1.94^{a}	13.63 ± 0.66^{a}
PDW (%)	8.7 ± 1.1^{a}	7.9 ± 0.10^{a}	8.3 ± 0.20^{a}	8.83 ± 0.11^{a}	8.6 ± 0.60^{a}
MPV (fL)	7.76 ± 0.35^{a}	7.23 ± 1.01^{a}	7.6 ± 0.00^{a}	7.16 ± 0.30^{a}	7.53 ± 0.05^{a}
PCT (ng/mL)	0.069 ± 0.025^{a}	0.078 ± 0.006^{a}	0.097 ± 0.063^{a}	0.096 ± 0.011^{a}	0.104 ± 0.004^{a}

Results were presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($P < 0.05$). WBC = White blood cell, LYMP = lymphocyte, MONO = monocyte, GRA = granulocyte, RBC = red blood cell, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red blood cell distribution width, PDW = platelet distribution width, MPV = mean platelet volume and PCT = plateletcrit.

and T_{Con}. The highest platelet distribution width was observed in T_{Pre} and the lowest in T_{Ant}. Mean platelet volume was highest in T_{Con} and lowest in T_{Pre}. Plateletcrit value was highest in T_{Syn} and lowest in T_{Con}.

3.4. Histological Analysis. Histological analysis of liver tissue structure is shown in Figure 2. Liver histology of all treatments shows normal hepatocyte structure with centrally located circular nuclei. There are no abnormalities in any liver tissue structure and many lipid vacuoles are present. The shape of the lipid vacuoles indicated glycogen accumulation in liver tissue.

3.5. Economic Analysis. Detail economic analysis is given in Table 5. Total variable cost and total cost varied significantly ($P < 0.05$) among treatments having higher costs in T_{Syn}. However, there was no significant ($P < 0.05$) difference in gross benefit and net benefit. There was a significant ($P < 0.05$) difference in the cost-benefit ratio and the highest value was observed in T_{Pro}.

4. Discussion

Tilapia is enormously popular in Asian countries because of its easy rearing process and faster growth [43, 48]. To ensure higher productivity, different types of antibiotic doses are used extensively without regulation. Sulfamethoxazole has

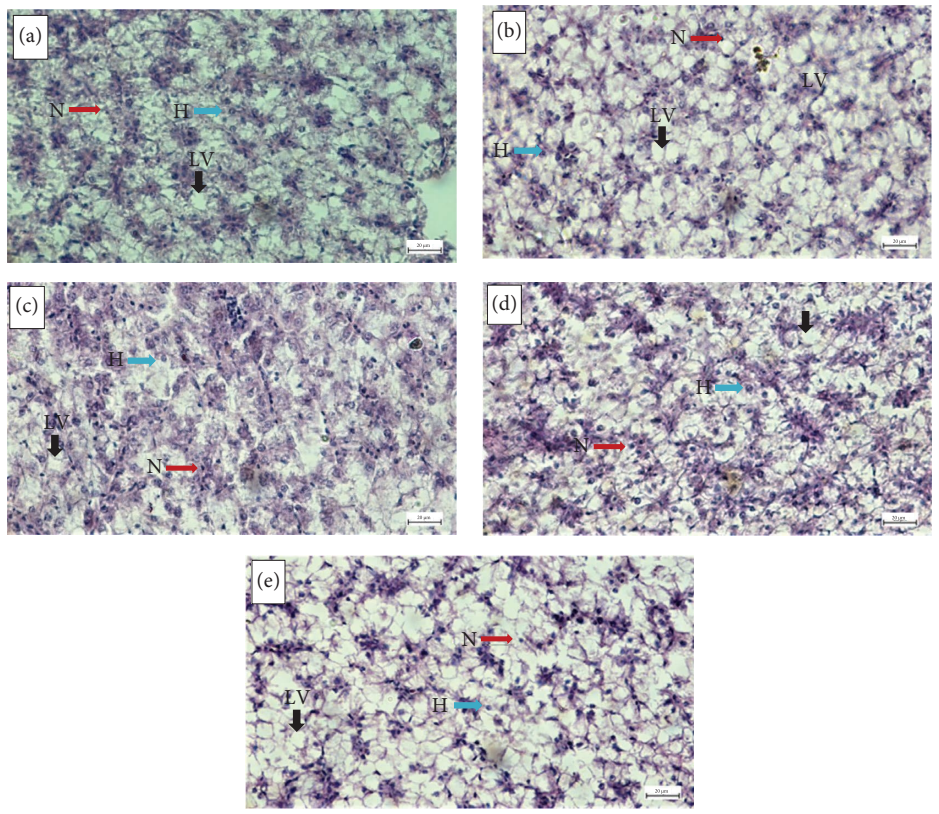

FIGURE 2: Histological images (×400) of the liver of Nile tilapia. The liver of the control diet showed normal histological structure. The liver of different treatments also showed the same normal histological structure of the control diet with increased hepatocytes (blue arrows), nucleus (red arrow), and lipid vacuoles (black arrow). (a) = control, (b) = antibiotic (cotrim vet), (c) = probiotic (pond care), (d) = prebiotic (spirulina), (e) = Commercial probiotic + prebiotic. The scale bar represents 20 μm.

TABLE 5: Economic analysis in different treatment groups.

Costs variables per treatment	Treatments				
	T_{Con}	T_{Ant}	T_{Pro}	T_{Pre}	T_{Syn}
Fry (US$)	0.24 ± 0.0^{a}	0.24 ± 0.0^{a}	0.24 ± 0.0^{a}	0.24 ± 0.0^{a}	0.24 ± 0.0^{a}
Feed (US$)	0.24 ± 0.0^{a}	0.24 ± 0.02^{a}	0.27 ± 0^{a}	0.29 ± 0.0^{a}	0.29 ± 0.0^{a}
Treatment cost (US$)	0 ± 0.0^{a}	0.001 ± 0.0^{a}	0.03 ± 0.0^{a}	0.15 ± 0.0^{a}	0.18 ± 0.0^{a}
Total variable charge (US$)	0.48 ± 0.01^{b}	0.48 ± 0.02^{b}	0.54 ± 0.01^{ab}	0.68 ± 0.0^{ab}	0.71 ± 0.0^{a}
Fixed cost	0.25 ± 0.0^{a}	0.25 ± 0.0^{a}	0.25 ± 0.0^{a}	0.25 ± 0.0^{a}	0.25 ± 0.0^{a}
Total cost (US$)	0.73 ± 0.0^{b}	0.73 ± 0.02^{b}	0.78 ± 0.0^{ab}	0.92 ± 0.0^{ab}	0.96 ± 0.0^{a}
Gross benefits (US$)	1.1 ± 0.02^{a}	1.1 ± 0.02^{a}	1.33 ± 0.02^{a}	1.34 ± 0.0^{a}	1.34 ± 0.0^{a}
Net benefits (US$)	0.38 ± 0.02^{a}	0.37 ± 0.02^{a}	0.55 ± 0.01^{a}	0.42 ± 0.0^{a}	0.38 ± 0.0^{a}
Cost-benefit ratio (BCR)	1.52 ± 0.02^{ab}	1.52 ± 0.05^{ab}	1.70 ± 0.02^{a}	1.45 ± 0.01^{b}	1.40 ± 0^{b}

Results were presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($P < 0.05$). Price of each fry = US$0.24; feed cost = 0.0012 US$/gram of feed; antibiotic cost = 0.024 US$/gram antibiotics; probiotics cost = 0.116 US$/gram probiotics; prebiotics cost = 0.012 US$/gram prebiotics; selling price = 0.012 US$/inch size tilapia fingerling.

been known to limit bacterial growth by interfering with bacteria's protein synthesis and protecting fish from bacterial invasion [49]. Antibiotics are not reported to improve growth or feed efficiency in fish [50], while excessive use of antibiotics may inhibit aquatic animal growth [51, 52]. The administration of probiotics enhances fish health status, immune response, and protection against several pathogens in fish [53]. The probiotics-supplied treatment gave a better growth rate, survival rate, and specific growth rate than the control diet [54–56]. In our current study, higher weight gained, specific growth rate, and lower food conversion values were observed in probiotics supplemented diet than control, which seems correlated with previous different findings.

Spirulina contains different polyunsaturated fatty acids, vitamins, and pigments that enhance fish growth and improve coloration [57]. Spirulina supplementation improved growth performance than control in our study, and this relates to the findings of Elabd et al. [58]. The treatment with spirulina supplementation showed a better growth rate, specific growth rate, and survival rate than the control diet in *Oreochromis niloticus* [59, 60], and these findings were related to current research. El-Araby et al. [61] evaluated that dietary supplementation of spirulina in Nile tilapia showed higher growth and feed efficiency ratio. Different results indicated that fish that provided synbiotics performed better [62]. The highest growth rate was observed in the synergism of commercial probiotic and spirulina-provided treatment in our study, and this correlates with the findings of Al-Deriny et al. [63] and Cavalcante et al. [64]. Therefore, current research also evidences that probiotics, prebiotics, and synbiotics treatment result in better growth performance and feed utilization than antibiotic treatment.

Hematological parameters are relevant indices of fish's physiological condition, environmental condition, disease, and effect of diet [65]. Red blood cell contributes to respiration and white blood cell and platelets work together to generate an immune response in fish. White blood cells provide immunity by protecting against microbial invasion [66]. Shen et al. [67] have found that red blood cells and white blood cells both generate an immune response in *Oreochromis niloticus*. Platelets contribute to immune responses by forming bonds with lymphocytes and monocytes and acting as immune cells [68]. Saglam and Yonar [69] found a decrease in white blood cell count in orally administered sulfamerazine in *Onchorhynchus mykiss* than control which matches our findings. Iftikhar and Hashmi [70] found an increase in white blood cell count in sulfamethoxazole-administered*Cyprinus carpio* which differed from our current study. Moustafa et al. [71] observed an increase in white blood cell and red blood cell counts with increasing *Bacillus* strain probiotic supplementation. Tachibana et al. [72] observed no significant difference in lymphocyte, monocyte, and red blood cell values in *Bacillus* strain provided by Nile tilapia. Telli et al. [73] found higher mean corpuscular hemoglobin concentration and platelets in probiotic bacteria *B. subtilis* supplemented in Nile tilapia. This result is similar to the platelet value in our study but different in mean corpuscular hemoglobin count. Elsabagh et al. [74] discovered that *Bacillus* strains mixture probiotic increased RBC, hemoglobin, lymphocyte, and monocyte counts in *Oreochromis niloticus*. This result is similar to all values observed except lymphocyte count in the current study.

Al-Zayat [75] observed elevated values for hematological parameters fed with 5% spirulina. Sherif et al. [76] found higher RBCs and WBCs in spirulina-provided diets in *Oreochromis niloticus*. Abdel-Tawwab et al. [42] found increased red blood cell and white blood cell counts with increased inclusion of spirulina in Nile tilapia. All these findings indicate enhancement of hematological indices provided by spirulina, which is supportive of our observed result. Both probiotic *Aspergillus oryzae* and prebiotic β-Glucan provided Nile tilapia hematological analysis found increased red blood cells and white blood cells with the inclusion of both treatments either separately or in synergism [20]. All the previous findings were aligned with the results of our research.

Hepatic histology seems to be an essential biomarker in determining the health status of fish [2, 77, 78]. Moustafa et al. [71] found a normal hepatic portion in *Bacillus*strain-provided Nile tilapia, and this correlates with our findings. Sulfamethoxazole is a fat-soluble antibiotic, and it increases triglycerides in fish liver, which causes fatty liver [79]. Different treatment units in the current study also followed and remained consistent with the previous research. However, production cost was recorded higher for probiotics, prebiotics, and synbiotics treatment than antibiotics. Probiotic had a higher cost-benefit ratio than other treatments indicating that probiotic is more economically beneficial. Probiotics, prebiotics, and synbiotics had a significant difference in growth performance and feed utilization parameters compared to antibiotics. Probiotics and prebiotics are also considered safe for the environment and other organisms and are known to improve water quality [80, 81]. Considering the health hazard of antibiotics and the beneficial effect of probiotics, prebiotics, and their synergisms on growth and feed utilization, they could be recommended to use as an alternative to antibiotics in aquaculture.

5. Conclusion and Limitation

Due to recent concerns about public health issues with antibiotics usage, probiotics, prebiotics, and synbiotics are the most reliable alternative as growth-enhancer and improvement of antimicrobial properties of the fish gut. The current study revealed that probiotics, prebiotics, and synbiotics had a positive effect on fish growth, hematology, liver histology, and improved feed utilization parameters compared to antibiotics. Therefore, it could be stated that probiotics, prebiotics, and synbiotics have the potential to replace antibiotics in aquaculture. However, still, a broad range of antibiotics, probiotics, and prebiotics treatment with different-sized and aged fishes will require setting up appropriate doses for aquaculture practices. The effectiveness of different administration methods also needs to be evaluated for better comparison.

Data Availability

Data used to support the findings of this study are made available from the corresponding author upon request.

Disclosure

The submitted article has not been published previously (except in any form of an abstract or a published lecture), and it is not under consideration for publication elsewhere.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Marjana Jannat Munni conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, and wrote the paper. Kazi Rabeya Akther and Shamim Ahmed analyzed and interpreted the data and wrote the paper. Mohammad Amzad Hossain conceived and designed the experiments, analyzed and interpreted the data, and wrote the paper. Nirmal Chandra Roy conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, contributed to reagents, materials, analysis tools or data, and wrote the paper.

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