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Do Dietary Synbiotics Modulate Growth Performance and Haematological Properties of Tilapia, *Oreochromis niloticus?*

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ABSTRACT

A 60-day long aquarium trial was conducted in laboratory condition to assess the growth performance of tilapia *Oreochromis niloticus* fed with various types of synbiotic diets. Twenty-five homogenous fry were randomly assigned into four different treatments with three replications. Fish were fed with experimental diets containing 0.3% probiotic (PBO)+1.5% molasses, 0.3% PBO+1.5% yeast and 0.3% PBO+1.5% sugar and marked as T_1 T_4 (control), T_2, T_3 and T_4 and fish in control were feed with commercial diet. Length and weight were recorded every 10 days and at the end of the trial blood samples were extracted for haematological analysis. The T_4 group showed significantly higher growth performance among the treatments and differences between T_1 and T_2 were found as non-significant ($P<0.05$). Again, significantly superior Specific Growth Rate (SGR %) was found in T_4 and non-significant differences detected between T_2 and T_3 as well ($P>0.05$). Haematological analysis showed significant changes among the treatments for hemoglobin, Red Blood Cell (RBC) count and White Blood Cell (WBC) count ($P>0.05$). All treatments showed better growth performance in contrast to the control group. Finally, present results indicate that synbiotics might increase the growth performances, haematological indices and health condition in *O. niloticus*.

Keywords: Synbiotics, probiotics, growth performance, haematological properties, *O. niloticus*

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Introduction

Tilapia, *Oreochromis niloticus* is deemed as one of the most promising species for commercial aquaculture all over the world (Akter et al. 2019; Goda et al. 2012). This fish shows good physical and environmental adaptability, greater relative resistance to handling stress, diseases, captivity and at the same time not compromising its flesh quality and growth (Alam et al. 2014; Das et al. 2019; Welker and Lim 2011). Growth performance of fish mostly depends on several biotic and abiotic factors as well as on the feed fed to the fish (Burel et al. 1996; Hossain et al. 2017). The rapid expansion of commercial Tilapia culture requires stable diets for better growth and physiological performance (Goda et al. 2012; Hasan et al. 2021).

More than 50% of the modern intensive aquaculture costs are based on feed costs (Ibrahem et al. 2010), consequently reduction in feed costs without affecting growth and flesh quality is now getting concern (Francis et al. 2005). In recent day aquaculture interest in probiotics has raised which helps in reduction of mortality rate (Denev et al. 2009; El-Haroun et al. 2006), improved the ability to antagonism in water body (Denev et al. 2009; Panigrahi et al. 2005) and rectify better fish growth and immune responses (Goda et al. 2012). Prebiotics are gut microbial elements which positively affect the host through stimulating the growth and acting on pathogenic bacteria (Ibrahem et al. 2010). The prebiotics are efficient in modification of gut intestinal microbial community, provide better nonspecific immune responses for protection (Bailey et al. 1991), boost up fermentation processes of digestive products (Smiricky-Tjardes et al. 2003), offer better mineral reception by body (Bongers and van den Heuvel 2003) and improve disease resistance (Bailey et al. 1991). The use of synbiotics i.e., both probiotics and prebiotics would be a revolutionary development leading to the formulation technique of modern aquatic feed industry (Dawood et al. 2020). Synbiotics are also termed as functional additives in diets formulated by combining probiotics and prebiotics (Hoseinifar et al. 2015). In spite of the short history of use of the synbiotics, they attracted great attention due to their synergistic effects (Dawood et al. 2018).

Materials and Methods

Designing The Experiment, Diet Preparation And Duration Of The Trial

The duration of this study was 60 days. During the experiment, rectangular shaped aquariums sized 30” ×15” ×15” were stocked by twelve fish fry each. Each aquarium was facilitated with aerator for ensuring the proper dissolved oxygen in the water. Electricity supply was facilitated from the electricity facility of the laboratory. A commercial tilapia fish diet (Quality fish feed) was used as a basal diet. The nutritional arrangement of the basal diet was recorded as Carbohydrate 25%, Protein 40%, Lipid 6%, Ash 10%, Fiber 5%, Moisture 11%, and Minerals 3%. Four experimental diets were prepared by adding different amounts of molasses/ yeast/ sugar along with commercial probiotic *Aquastar grow-out* (Manufactured by Renata Animal Health) to the basal diet at a level of 0% (T_1 control), 1.5% molasses and 0.3% probiotic (T_2), 1.5% yeast and 0.3% probiotic (T_3) and 1.5% sugar along with 0.3% probiotic (T_4) (Table 1). After the acclimatization of tilapia fry to the laboratory condition, homogeneous sized fry were sorted and each of the experimental glass aquarium which were stocked with 25 fries following completely randomized design. Fish were fed thrice a day with high protein commercial powder feed at a rate of 5% body weight. Uneaten feed was removed through siphoning with 50% water renewal at two days interval.

Table 1. Design of the experiments and dosage

Treatment	Dose of Synbiotics	Feed Fed (% body weight)	Stocking Density	Replication
T_1 (control)	0%	5%	25	3
T_2	0.3% probiotic +1.5% molasses	5%	25	3
T_3	0.3% probiotic +1.5% yeast	5%	25	3
T_4	0.3% probiotic +1.5% sugar	5%	25	3

Sampling Fish And Observing Water Quality Parameters

The water parameters were monitored before using and during using the tap water in aquaria. The water temperature, pH, dissolved oxygen (mg/l), NH_3 (mg/l) were measured using thermometer, pH meter (Hanna Instrumental, manufactured in Japan), DO meter (YSI 58), Ammonia test kit (Model HI 3824, Hanna Instrumental Company, Japan) respectively. Fish were sampled every 10 days in the study during the 60 days period. Weight and length were measured through the numeral balance (CAMRY, EK 3052) and a scale tape, respectively.

Collection And Analysis Of Blood Samples

Following the completion of the trial, fish were left starving overnight and sedated with eugenol at 50 mg/L before sampling blood. A 1 ml hypodermal syringes were used for the collection of blood from the fish. Blood was extracted from the caudal vein of five and transferred to EDTA tube immediately to avoid coagulation of the blood. Blood parameters were analyzed in central laboratory of Veterinary, Animal and Biomedical Sciences faculty by using CBC analyzer.

Tools of growth parameters

Different growth parameters were calculated by using the mathematical terminologies from the previous description of Panase and Mengumphan, (2015), Pechsiri and Yakupitiyage, (2005) and Olvera-Novoa et al. (1990).

$$WG = MVFW - MVIW$$

(WG = Weight Gain, MVFW = Mean Value Of Final Weight, MVIW = Mean Value Of Initial Weight)

$$WG(\%) = (W2 - W1)/W1 * 100$$

(W2 = Final Weight Of Fish, W1 = Initial Weight of fish)

$$SGR(\%) = (W2-W1)/(T2-T1)$$

(SGR = Spesific Growth Rate, T2,T1 = Duration in days)

$$LG = L2 - L1$$

(LG = Length gain, L2 = Mean Value Of Final Length, L1 = Mean Value Of Initial Length)

$$LG(\%) = (L2 - L1) / L1 * 100$$

DWG = (MVFM – MVIW) / T3
(DWG = Avarage Daily Weight Gain, T3 = Duration Of Experiment In Days)
DLG = (L2 - L1) / T3
(DLG = Avarage Daily Length Gain)

The values of Fulton's condition factor (K) was estimated by plotting length weight data on the following equation adopted from Htun-Han, (1978).

$$K=(W*100)/L^3$$

Statistical Analysis

The analysis of different growth and blood parameters were performed by using one-way analysis of variance (ANOVA) at P<0.05 in IBM SPSS program version 26.

Results

Regular monitoring of physicochemical parameters during the experimental period was done to maintain the suitable growth performance of Tilapia. The temperature, pH, dissolved oxygen and NH_3 were monitored regularly and recorded. Results showed that the water qualities were suitable for the development of Tilapia (Table 2).

Table 2. Mean values of water quality parameters

	T_1	T_2	T_3	T_4
Temperature (°C)	26.13±1.08[a]	26.17±1.12[a]	26.37±0.71[a]	26.15±1.09[a]
Dissolved Oxygen (mg/L)	5.49±0.20[a]	5.53±0.21[a]	5.55±0.17[a]	5.47±0.15[a]
pH	7.19±0.03[a]	7.2±0.05[a]	7.21±0.06[a]	7.18±0.04[a]
NH_3 (mg/L)	0.014±0.00[a]	0.012±0.00[a]	0.014±0.00[a]	0.013±0.00[a]

The growth parameters were measured at the intervals of 10 days. The highest mean weight gain was observed in T_4 (17.76±0.55 gm) followed by T_3 (11.42±0.73 gm) and T_2 (9.03±0.42 gm). The lowest mean weight gain was observed in T_1 treatment 78.53±0.48 gm (Table 3). Results demonstrated that the weight gain of the sugar group rose significantly among treatments whereas the weight gain decreased in the yeast group which was significantly better than control and molasses groups (P<0.05). No significant variations were found between the control and molasses groups (P>0.05). The mean percentage weight gain was found maximum in T_4 treatment 1531.07±83.84 which was followed by T_3 (681.73±16.97) and T_2 (585.46±23.87) (Table 3). Mean specific growth rate (%) in T_4 was found maximum 2.64±0.12 and it was followed by T_3 (1.73± 0.07) and T_2 (1.63±0.09) (Figure 1). The lowest percentage weight gain (%) and specific growth rate (%) was observed in T_1 treatment 536.48±18.21 and 0.80±0.05 respectively (Table 3; Figure 1). No significant changes were found in PWG and SGR of T_2 and T_3 (P>0.05) whereas the T_4 showed significantly higher PWG and SGR. It was observed that the lowest PWG and SGR was in T_1 control group. Significant length gain was observed in each treatment. Lowest mean length gain was observed in T_1 treatment whereas significantly better length gains were observed in T_2, T_3 and T_4. Highest mean length gain was observed in T_4 which was significantly higher than any other treatments (P<0.05). T_4 showed the maximum result 7.18±0.11cm followed by T_3 and T_2. Lowest mean length gain was observed in T_1 treatment 3.49±0.11 cm (Table 3).

Table 3. Growth parameters of Tilapia for 60 days experimental period

Parameters	T_1	T_2	T_3	T_4
Mean Initial Weight (g)	1.59±0.17[a]	1.66±0.14[a]	1.73±0.14[a]	1.32±0.12[a]
Mean Final Weight (g)	10.12±0.69[a]	10.69±0.55[a]	13.15±0.86[b]	19.07±0.65[c]
Mean Weight Gain (g)	8.53±0.48[a]	9.03±0.42[a]	11.42±0.73[b]	17.76±0.55[c]
Weight Gain (%)	536.48±18.21[a]	585.46±23.87[a]	681.73±16.97[b]	1531.07±83.84[c]
Mean Initial Length (cm)	4.43±0.09[a]	4.45±0.11[a]	4.44±0.11[a]	4.38±0.14[a]
Mean Final Length (cm)	7.92±0.23[a]	8.88±0.27[a]	10.71±0.25[b]	11.56±0.29[c]
Mean Length Gain (cm)	3.49±0.11[a]	4.43±0.16[b]	6.27±0.15[c]	7.18±0.11[d]
Length Gain (%)	78.78±1.74[a]	99.02±1.58[a]	141.63±2.33[b]	163.11±3.54[c]
Survivability Rate (%)	100	100	100	100

The maximum mean percentage length gain was found in T_4 treatment which was significantly higher among treatments ($P<0.05$). The lowest PLG was found in T_1 78.78±1.74 as well (Table 3). The condition index was found to be less fluctuating among the different treatments, however the highest value occurred at T_4 whereas the lowest at T_1 ($P<0.05$) (Figure 1).

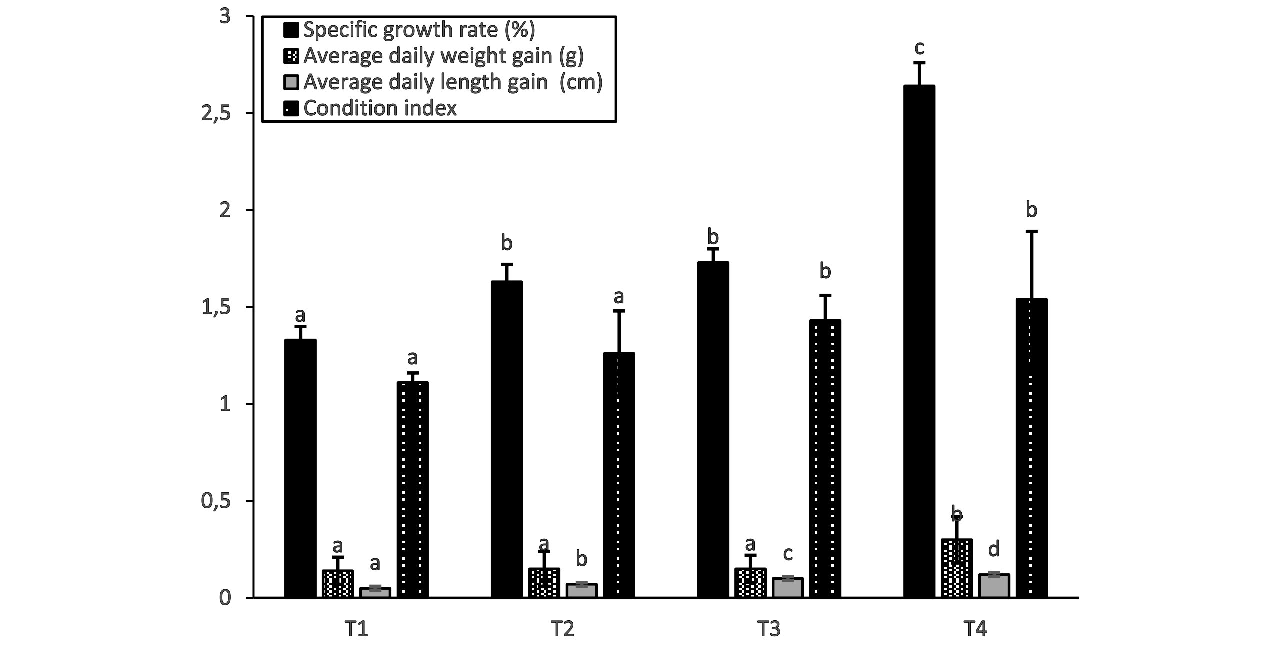

Figure 1. Specific growth rate (SGR %), average daily length gain (cm), average daily weight gain and condition index of tilapia during the 60 days experimental period.

The lowest hemoglobin concentration (g/dL) was found in T_1 while the maximum hemoglobin concentration was observed in T_4 with a gradual decrease in T_3 and T_2 ($P<0.05$) (Figure 2.A). Insignificant WBC count was observed in T_3 and T_4. In contrast to the control group significant increases were found in T_2, T_3 and T_4 (Figure 2.B). The mean value of RBC was found higher than control group in other treatments. Amongst the treatments the lowest RBC counts were observed in T_1 control groups 0.98±0.07 m/µl which were significantly lower among treatments ($P<0.05$) (Figure 2.A).

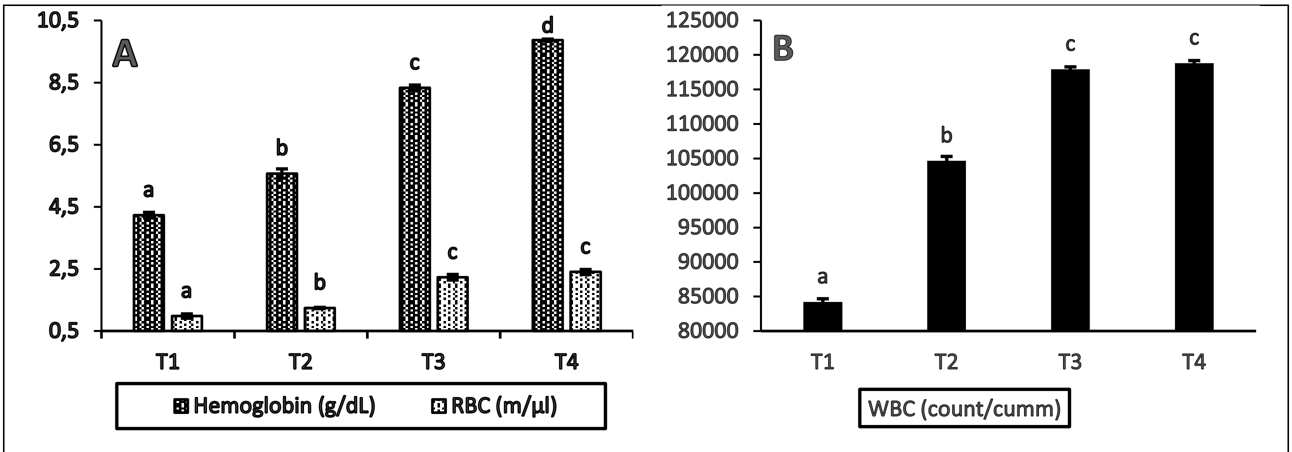

Figure 2.A-B Blood parameters of Tilapia during the experimental period (*WBC= White blood cell, RBC= Red blood cell, g/dL= gram per deciliter, count/cumm= count per cubic millimeter, m/µl= millions per micro liter).

Discussion

Growth of fish and other biological activities are largely dependent on water quality parameters (Ahmed et al. 2020). The mean temperature recorded in this experiment was 29±0.91°C. The temperature range from 20°C to 36°C has been reported to be optimal for most tropical fishes (Kausar and Salim 2006; Ngugi et al. 2007). Optimum level of dissolved oxygen is crucial for the growth, development and survival of fish and DO more than 5 ppm is crucial to support fish growth and reproduction (Bhatnagar and Singh 2010). The mean DO level during the experimental period was 5.64±0.05 ppm. The mean value of pH during this experiment was observed as 7.19±0.01. Highly acidic water with less than pH 5.5 can suppress the growth and reproduction of fish and an ideal pH range should be between 6.5 and 7.0 though it is also considered that pH ranging from 6.1 to 8.0 is reasonable for the survival and growth of fish (Crane 2006: Bryan et al. 2011). The maximum ammonia tolerance level in water is ranged as 0.1-0.2 mg/L for most of aquatic animals (Bhatnagar and Devi 2013; Santhosh 2017). Therefore, the recorded water quality factors during this research were found within the appropriate level for Tilapia culture.

The growth performance of tilapia is positively related with the amount of fed synbiotics in this experiment. Diets fed in this experiment containing the supplements of probiotic, yeast, sugar, and molasses for different treatments showed better growth performances than control groups. The significant increase in growth performances of beluga, *Huso huso* fed with dietary supplementation of *Saccharomyces cerevisiae* (Hoseinifar et al. 2011a; Ta'ati et al. 2011). Akrami et al. (2013) was observed compared to the control groups. The higher growth and lower FCR was also observed for stellate sturgeon *Acipenser stellatus* fed with fructo-oligosacharides. In the current experiment the fish fed with different synbiotics showed better growth performances and health status, which is similar to the conclusions of Hoseinifar et al. (2011b). Other researchers also found better results than the control using different synbiotics in feed (Reza et al. 2009; Hoseinifar et al. 2011c; Mansour et al. 2012).

The better growth performances found in the fish fed with synbiotics may be due to synbiotics effect on enhancement of the survival and flourishment of live microbial nutritive additives in the digestive tract through triggering better metabolism health promoting bacteria (Montajami et al. 2012; Nekoubin et al. 2012). Among the synbiotics treatments, current study revealed highest growth performances in T_4 and T_3 which is significantly better than control and T_2 treatment. Ozório et al. (2012) carried out an experiment with dietary yeast at a level of 0, 10, 15, 20, 30 and 40% inclusion in feed and found better growth and physiological performances in the lowest level of yeast inclusion in feed. Zhou et al. (2018) mentioned that dietary yeast is a precious source of immune promoting complexes and it was demonstrated to positively impact the growth increment, immune response, or anti-stress response of different aquaculture species (Andrews et al. 2011; Li and Gatlin 2005). Fish of T_3 treatments fed with 1.5% yeast along with 0.3% probiotics respectively showed better growth performances than the control and T_2 treatment. This may be due to the collective action of yeast and probiotic as growth promoters in fish. Though the level of yeast inclusion was lower than the observation of Ozório et al. (2012), it showed better growth and physiological performances in tilapia. Some researchers also mentioned that the supplementation of dried yeast at low levels efficiently increased growth of fish (Rumsey et al. 1991; Oliva-Teles and Gonçalves 2001; Mc-Lean and Craig 2006). Goran et al. (2017) reported that the dietary inclusion of yeast enhances the growth and haematological parameters in common carp. Anderson et al. (1984) reported that carbohydrates can have value as energy supplier for tilapia. In this experiment sugar and molasses were used with probiotics to determine the growth performance of tilapia. The results obtained from the sugar treatment showed maximum growth performance in fish fed with molasses compared to the controls. Several studies reported that high carbohydrate level might depress the growth rate and may enhance mortality rate (Phillips et al. 1948; Austreng et al. 1977). Among the diets such as T2 and T4 consisting of synbiotics and carbohydrate inclusions, fish in T_4 treatment, which was formulated with 1.5% table sugar and 0.3% probiotics, showed the maximum growth performance.

Some studies mentioned that fish fed with polysaccharides showed better growth than fish fed with monosaccharides or di-saccharides (Lee et al. 2003; Lee and Lee 2004; Tan et al. 2006) which was also observed in several studies. (Hung and Storebakken 1994; Enes et al. 2008. In the current experiment the best growth and other haematological parameters were observed at maximum level in sugar treatment than other treatments. This can be traced to the fact that molasses contain a combination of sucrose, fructose and glucose and they are more complex than table sugar to absorb in body, whereas the glucose is known to hinder the carriage of amino acids at absorption spots on mammalian's bodies (Alvarado 1966; Alvarado and Robinson 1979). More recently similar consequence was documented in fish (Hokazono et al. 1979). In this experiment this may explain the effect on growth performance and the lowest results among the fish fed with synbiotics. Similar results were observed by Shiau and Chuang (1995) where they found that sucrose fed fish showed better growth performances such as weight gain, FCR than glucose in tilapia. The growth performances of T_3 treatment were also satisfactory compared to the control and molasses groups. This may be because yeast itself play a role as a probiotic (Pandiyan et al. 2013) and prebiotic together and it is very helpful for the propagation of some probiotic bacteria (Zhou et al. 2018). The WG, LG, PWG, PLG and SGR of tilapia fed with synbiotics increased in relation to the control group. Similar results were observed by Hassaan et al. (2014) during their experiments using synbiotics fed to tilapia. A research by Ye et al. (2011) stated the Japanese flounder fed on experimental diet supplemented with fructo-oligosaccharides (FOS), oligosaccharides (MOS) and live *Bacillus clausii* boosted weight gain. Ai et al. (2011) suggested that at each nutritional fructo-oligosaccharides level additionally accompanied with *B. subtilis* considerably increased specific growth rate percentage and feed efficiency ratio (FER) while equated with control group for young yellow croaker, *Larimichthys crocea*. Zaid et al. (2013) reported superior growth performances of *Clarias gariepinus* increased at 1.5% inclusion of molasses in feed and this experiment is lined with this finding as it showed better growth performance in tilapia fed with molasses compared to the control group.

The nature of dietary composition, stress and water toxicity affect the haematological parameters and fitness of fish (Chen et al. 2004; Wörle-Knirsch et al. 2007). Hemoglobin reading is important to determine the consequence of stressors on the fish health (Munikittrick and Leatherland 1983). Hemoglobin level of tilapia in this experiment was found significantly increased compared to the control. Hemoglobin is a complex molecule in oxygen delivery system and it is established as a good marker of anemic illnesses in fish (Giardina et al. 1973; Riggs 1970). The recorded value in current experiment showed that fish were not hurt from any form of anemic illness. The maximum hemoglobin level was found in the sugar treatment, which is followed by yeast and molasses treatment respectively. All treatments showed significant increases of hemoglobin in this experiment. The white blood cells count showed a significant increase with the treatments and the maximum was in the sugar treatment. Compared to the control group WBC count was observed significantly higher which is in line with the outcomes of Zaid et al. (2013). Biswas et al. (2012) indicated that dietary yeast extract activated different functions of leucocytes which are kind of WBC, including phagocytosis that resulted an increase in phagocytic value index and number of WBC. In this experiment similar results were obtained from the yeast treatment and there may be similar reason behind the increase of WBC in the synbiotics treatment. Value of RBC in this experiment increased significantly compared with the control group and it was observed that the hemoglobin, white blood cells and red blood cells decreased from sugar treatment toward controls which was similar to the growth performance results of this experiment. The findings of white blood cells and red blood cells seems to be aligned with the outcomes from Hassaan et al. (2014), in whose studies they noticed the value of white blood cell and red blood cell increased when their growth performances were maximum. Finally, it can be concluded that synbiotics have constructive effects on growth and haematological parameters of Tilapia in comparison to the control treatment and diets which included synbiotics (1.5% sugar+0.3% probiotic) were the best diets in the present study. In the present research, the results revealed that the diets including synbiotics have significant impacts on the growth of tilapia. It can be summarized that the feed containing 0.3% probiotic and 1.5% sugar is the most diet suitable for the better growth performance and hematological parameters among all treatments. Other synbiotics treatments also showed better growth and hematological performance than control group. Further research needs to be carried out to analyze the appropriate effects of synbiotics and other fish trials are also suggested.

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