

Effect of Different Dietary Probiotics and Prebiotics on Growth, Feed Utilization and Digestive Enzymes Activities of Nile Tilapia, *Oreochromis niloticus* Fingerlings

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Abstract: A 70 days feeding trial was conducted to investigate the effects of two dietary probiotics; *Bacillus subtilis* KCTC 2217 or *Bacillus licheniformis* KCCM 11775 with two prebiotics; mannan oligosaccharide (MOS) or fructooligosaccharide (FOS) on Growth performance, Feed Utilization, economic evaluation, chemical body composition and Digestive Enzymes Activities of Nile tilapia fingerlings. Fish averaging 10.8 ± 0.50 g (mean \pm SD) were randomly distributed into five treatments with triplicate tanks. A basal control diet (CON) and four synbiotic diets supplementing *B. subtilis* + MOS (BSM), *B. subtilis* + FOS (BSF), *B. Licheniformis* + MOS (BLM) and *B. licheniformis* + FOS (BLF). Results showed that dietary *B. subtilis* with FOS (BSF) and *B. licheniformis* with MOS (BLM) give the best weight gain and specific growth rate than other treatments. In general, weight gain and specific growth rate of fish fed all synbiotic diets were higher than fish fed control diet. It could be concluded that dietary *B. subtilis* with FOS (BSF) and *B. licheniformis* with MOS (BLM) could have beneficial effects on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus*) fingerlings. These probiotics improved the fish enzyme activities of amylase in the gastrointestinal tract than the fish fed control diet; however, the protease and lipase activities were not affected. The present results recommend the incorporation of probiotics to Nile tilapia feed as supplements to stimulate fish growth and digestion.

Keywords: *B. subtilis*, *B. Licheniformis*, MOS, FOS, growth, digestive enzymes, Feed utilization.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*), which is characterized by rapid growth, high production, and good disease resistance, has become one of the most important freshwater aquaculture species in Egypt and the world (Yuan *et al.*, 2020). Tilapia production has significantly increased over the past few years due to the adoption of semi-intensive and intensive aquaculture technologies (Mugwanya *et al.*, 2021). Probiotic use in tilapia production is considered a viable, safe, and environmentally friendly strategy to enhance growth performance, feed utilization, immunity, disease resistance, and survival against pathogens and environmental stress. Hence, supplementation of fish diets and rearing water with probiotics can improve fish health (Mugwanya *et al.*, 2021). To date, many different types of probiotics, including lactic acid bacteria, *Bacillus* spp. and yeast, have been applied in aquaculture to promote the growth, feed utilization and gut health of tilapia (Elsabagh *et al.*, 2018). LAB, which are widely and effectively used in human and veterinary medicine, are classified as "generally regarded as safe" and have also been widely used in aquaculture, as a promising strategy for the cultivation of various species (Ringø *et al.*, 2018). Since LAB do not produce spores, unlike *Bacillus* spp., it is simpler to lessen the microbial load if necessary. Additionally, studies have shown the probiotic potential of LAB, such as *Lactobacillus plantarum* and *Lactobacillus lactis*, which are said to benefit fish survival following exposure to *Aeromonas hydrophila* and *Streptococcus Agalactiae* by enhancing growth and improving innate immunity (Yu *et al.*, 2017 and Xia *et al.*, 2020). An acceptable definition of 'probiotic' in aquaculture was given by Verschuere *et al.*, (2000), They defined probiotic as any live microbial adjunct that has a beneficial effect on the host by changes in the host-related or ambient microbial

community, through an improvement in the use of feed or its nutritional value, or by enhancing the host response to disease or by improving the quality of its environment. Currently, the most common probiotics used in aquaculture belong to *Lactobacillus* sp., *Bifidobacterium* sp., *Vibrio* sp., *Saccharomyces* sp., *Enterococcus* sp., *Bacillus subtilis* and *Bacillus* sp. (Kumar *et al.*, 2006); which are administered by enrichment of live foods, added to the diet or to the culture water (Panigrahi *et al.*, 2005). In the recent years, the demand for fish and other aquatic organisms increased for being a good source of animal protein and having a high nutrition value. Additionally, year by year the global and local production of fisheries decrease, therefore aquaculture in Egypt is a main source of fish production and represent about 80% of the local production according to (GAFRD, 2018 and FAO, 2020). Due to the continuous increase in population growth, the fish production must be directly proportional with this increase, but Egyptian aquaculture sector faces many challenges that hinder its development such shorting fresh water, increasing the feed price. Therefore, maintaining the quality of the cultured water for the longest possible period of time with raising the ability of fish to resistance the toxicity of water pollution and increasing their levels of heavy metals with improving the efficiency of fish feeding are one of the important solutions (Abdel-Aziz *et al.*, 2020). Using the micro-organisms is one of the most promising ways to improve water quality compared to other traditional methods. Prebiotic, probiotic, and synbiotic use in tilapia production is considered a viable, safe, and environmentally friendly alternative that enhances growth performance, feed utilization, immunity, disease resistance, and fish survival against pathogens and environmental stress. Their inclusion in fish diets and or rearing water improves the general wellbeing of fish. this review use of probiotics, prebiotics, and synbiotics and their effect on survival, growth, growth performance,

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gut morphology, microbial abundance, enzyme production, immunity, and disease resistance in tilapia aquaculture, while highlighting several hematological, blood biochemical parameters, and omics techniques that have been used to assess fish health. Furthermore, gaps in existing knowledge are addressed and future research studies have been recommended (El-Saadony *et al.*, 2021).

The aim of this study is to examine the impact of using the different types of microorganisms and prebiotics on parameters of water quality, growth performance, and hematological characters of Nile tilapia.

MATERIALS AND METHODS

This experiment was conducted in Fish Research Lab–Faculty of Agriculture, Suez Canal University – Ismailia-Egypt.

2.1 Experimental diets:

Dietary formulation and proximate composition of the basal diet are shown in (Table 1). Five experimental diets were formulated to be isonitrogenous and isoenergetic (300 g/kg crude protein and 470 Kcal/100g). A basal diet was used as control diet (CON.), and four other synbiotic diets were supplemented with two probiotics (1.0×10^8 CFU/g diet) with two prebiotics (Mannan and fructooligosaccharide) (5 g/kg diet); *B. subtilis* and Mannan (BSM), *B. subtilis* and fructooligosaccharide (BSF), *B. licheniformis* and Mannan (BLM) and *B. licheniformis* and fructooligosaccharide (BLF). Fish meal and wheat gluten meal were used as the major protein sources, soybean oil and fish oil as the lipid sources and corn starch as the carbohydrate source in the experimental diets.

All the feed ingredients were sampled for proximate analyses before feed formulation and the formulated experimental diets were analyzed to determine their nutritional composition (Table 1). All biochemical analyses were done on dry matter basis using standard methods of the Association of Official Analytical Chemists (AOAC, 2019). *Bacillus subtilis* and *B. licheniformis* were previously purchase from Commercial feed additives: Pro- Pac is a commercial probiotic produced by Pro-Byn International, Inc, USA and distributed by Nutrient animal health, Maddy, Cairo, Egypt. Which were used in this experiment. Mannan oligosaccharide and fructooligosaccharide were obtained from Elgamhoria Company.

2.2 Experimental fish and feeding trial:

The feeding trial was carried out at the fish research center–Faculty of Agriculture- Suez Canal University. Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from the fish research center–Faculty of Agriculture- Suez Canal University - Ismailia Governorate. At the start of the experiment, 15 Nile tilapia fingerlings with an initial weight averaging 10.50 ± 0.20 g (mean \pm SD) were randomly distributed into each of the 15 tanks with 25 L volume (3 replicates of 5 treatments) receiving a constant flow (1.2 L/min) of filtered freshwater. Every tank was supplied with air stone for continuous aeration. Fish were fed twice daily

(9:00 and 15:00 hr.) to apparent satiation for 70 days. Dead fish were removed immediately and weighed, and the amounts of feed for the respective tanks were adjusted accordingly. Uneaten feeds were siphoned out after 2 hr. of feeding.

Through the experimental period, temperature and dissolved oxygen were measured using Hach HQ40d meter (Hach Korea Inc.) twice daily (8:00 and 17:00 hr) in fish tanks. Water temperature and dissolved oxygen were measured every other day using an YSI model 58 oxygen meter (YSI Company, Yellow Springs Instrument, and Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). pH was monitored twice weekly using a pH meter (Orion pH meter, Abilene, Texas, USA) (APHA, 2005). Water temperature ranged from 26.17 ± 0.8 to 27.40 ± 0.30 °C: Dissolved oxygen, 6.23 ± 0.03 to 6.62 ± 0.44 mg L⁻¹. Total ammonia $0.010 \pm .005$ to 0.018 ± 0.12 mg L⁻¹ and pH 6.9 ± 0.14 to 7.40 ± 0.13 . Water quality criteria were suitable and within the acceptable limits for Nile tilapia *Oreochromis niloticus* fingerlings (Boyd and Massaut, 1999).

2.3 Sample collection and analysis:

At the end of the feeding trial, the total number and weight of fish in each tank were determined for calculation of weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), survival rate (SR), hematomatic index (HSI) and visceralsomatic index (VSI). Four fish per tank were randomly captured, anaesthetized with ethylene glycol phenyl ether (200 mg/L for 5–10 min), and the serum was separated by centrifugation at 5,000 g for 10 min and stored at -20°C for the analysis of blood biochemical parameters including glutamic pyruvic transaminase (GOT), total protein (TP) serum glucose (GLU), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes. Two additional fish from each tank were submitted to proximate composition analysis. Proximate composition analyses of the experimental diets and fish bodies performed by the standard methods of AOAC (2019) Official Methods of Analysis (16th edn.). Samples of diets and fish were dried at 105°C to a constant weight to determine their moisture content.

Ash content was determined by incineration at 550°C . Protein was determined using the kjeldahl method ($N \times 6.25$) after acid digestion, and crude lipid was ascertained by Soxhlet extraction using the Soxhlet system 1046 (Tacator AB) after freeze-drying the samples for 20 hr. and crude fiber was measured by ankomet test. Fish were weighed with a digital balance (0.01g) (model KERN 572-33, Germany). Fish were returned to their respective tanks after weight. At the end of the experimental period, fish were deprived of feed for 24 hours; all the fish were harvested, counted and weighed individually.

Table (1): The composition and proximate analyses of Experimental diets

| Ingredients | CON | BSM | BSF | BLM | BLF |
|---|--------|--------|--------|--------|--------|
| Fish meal | 4 | 4 | 4 | 4 | 4 |
| Gluten | 20 | 20 | 20 | 20 | 20 |
| Soybean meal | 25 | 25 | 25 | 25 | 25 |
| Wheat bran | 7 | 7 | 7 | 7 | 7 |
| Corn | 34 | 34 | 34 | 34 | 34 |
| Vit and Min. Mix ¹ | 2 | 2 | 2 | 2 | 2 |
| Fish oil | 3 | 3 | 3 | 3 | 3 |
| Soybean oil | 3 | 3 | 3 | 3 | 3 |
| <i>Bacillus subtilis</i> and <i>B. licheniformis</i> (g /kg) | 0 | 10 | 10 | 10 | 10 |
| <i>Manan and frac</i> (g /kg) | 0 | 5 | 5 | 5 | 5 |
| (CMC) Carboxy Methyl Cellulose | 2 | 2 | 2 | 2 | 2 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Approximate chemical analysis | | | | | |
| Dry matter (DM) % | 91.27 | 90.92 | 90.82 | 90.68 | 89.76 |
| Crude Fiber (CF) % | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| Crude protein (CP) % | 30.09 | 30.05 | 30.03 | 30.01 | 30.3 |
| Ether extract (EE) % | 12.99 | 13.38 | 13 | 13.22 | 13.1 |
| Ash% | 8.32 | 6.89 | 7 | 8.18 | 6.43 |
| NFE% ² | 41.9 | 42.98 | 43.27 | 41.89 | 43.47 |
| GE(Kcal/100g) ³ | 470.51 | 479.11 | 476.2 | 472.53 | 473.13 |
| DE(Kcal/100g) ⁴ | 352.88 | 359.33 | 357.15 | 354.40 | 347.24 |

1. Vitamin and mineral mixture/kg premix: Vitamin D, 0.8 million IU; A, 1.33g; D3, 1.68g; E, 6.66g; C, 16.8g; k, 0.8g; B1, 0.4g; Riboflavin 3.75g; B6 2.45g; B12, .33mg; NI, 9.42g; Pantothenic acid, 12.42g; Folic acid, 0.68g; Biotin, 16.6mg; BHT, 0.5g; Mn, 14.7g; Zn, 31.6g; Fe, 18.3g; I, 0.62g; Selenium, 0.22g and Co, 6.8mg.

2. Calculated by differences [Nitrogen free extract (NFE) = [100-(CP+ EE+ CF+ Ash)].

3. Gross energy value was calculated from their chemical composition, Estimated according to **Jobling, (1983)**. As 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

4. Digestible energy, estimated according to **Jobling, (1983)**, using digestible energy = gross energy X 0.75.

Fish performances under different treatments were evaluated in terms of final weight (g), daily weight gain (DWG, g day⁻¹), weight gain, specific growth rate (SGR, % day⁻¹), survival (%) and feed conversion ratio (FCR).

2.4. Measured parameters

2.4.1-Growth parameters:

Fish Weight gain (WG), Weight gain % (WG %), Specific growth rate (SGR) and Survival rate% (SR) were calculated using the given formula below:

- Weight gain (WG) = Final body weight (g) - Initial body weight (g).
- Weight gain % (WG %) = (Weight gain (g) / Initial body weight (g)) × 100.
- Specific growth rate % (SGR) = [(Ln FBW - Ln IBW) /day of experiment] × 100. (Where: FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic).
- Survival rate % (SR) = (Final number of fish / Initial number of fish) × 100.

2.4.2. Feed utilization parameters:

Feed intake (g/fish) is the amount of feed given or supplied during the experimental period for each fish per gram, and used for calculating the following equation:

- Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g)
- Feed conversion efficiency (FCE) = Weight gain (g)/ Feed intake (g)
- Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)
- Hematosomatic index (HSI, %) = (liver weight/ body weight) × 100.
- Visceralsomatic index (VSI, %) = (viscera weight/ body weight) × 100.

2.5. Fish body composition analysis:

At the beginning of the experiment, a pooled sample of three fish was taken randomly from the experimental batch of fish to serve as an initial carcass composition sample. At the end of the experiment, a random sample of three fish was collected from each replicate for the

final body composition analyses. The analysis of dry matter was done by drying pre-weighed samples in an oven at 105°C for 16 hours to reach a constant weight. The proximate analysis for crude protein (CP), crude lipids (CL), moisture and ash were carried out according to the standard methods by AOAC (2019).

2.6. Blood sample collection:

At the end of trial, three fish were sampled randomly from each replicate (4 samples per treatment) for immunological analysis (Fig 2). The fish were anaesthetized using clove oil (20 mg L⁻¹) of water. Blood samples (1 ml from each fish) were drawn from the caudal vein of each fish using a sterile syringe, previously rinsed with 2.7% Ethylene-diamine-tetraacetic acid (EDTA) solution as an anticoagulant (Plate 4). Blood samples were collected and processed according to standard methods described by Svobodová *et al.* (1991). The blood samples were used immediately for analysis of haemoglobin, red blood cells (RBC) and white blood cells (WBC). Extra blood sample (2 ml from each fish) were collected without anticoagulant and allowed to clot for 2 hours in Eppendorf tubes and centrifuged at 3000 rpm using an Eppendorf centrifuge (Centrifuge 5415 R®) for 10 minutes (Plate 5). Blood serum was collected from each centrifuged sample with a micropipette and stored at -20°C in Eppendorf tubes for analysis of serum Hematological parameters.

2.7. Hematological parameters:

The serum levels of TP, GLU and GOT were measured by a chemical analyzer (Fuji DRI-CHEM 3500i, Fuji Photo Film, Ltd.). Biochemical blood analyses consisted of the determination of glucose (mg/dL), total proteins (g/dL). Estimate the plasma AST and ALT activity using a colorimetric method of (Reitmen and Frankel, 1957).

2.8. Preparation of crude enzyme extract:

After the feeding trials, the tissue homogenates were prepared as described by Alarcón *et al.* (1999) with slight modifications. In a nutshell, the fish were starved for 12 hours before sampling, then quickly murdered by cold shock and dissected. To get rid of feed residue, the stomach contents were squeezed out and cleaned with cold distilled water. Ten percent (w/v) tissues homogenate was made using an electric homogenizer (KEMI (P) Ltd, Ernakulam, India, and Model No. KHH 1) in ice-cold Tris-HCl 50 mM buffer pH 7.2. The homogenate was then centrifuged for 10 minutes at 4 °C and 10,000 g. The supernatant containing the enzymes was stored at -20 °C, and this is used for all the analysis. The tissue homogenates were purified by 10% trichloroacetic acid (TCA) precipitation, and the precipitate of soluble protein was dissolved in 0.1 m NaOH. The soluble protein content of enzyme extract was measured according to Lowry *et al.* (1951). All spectrophotometric analysis was carried out using Hitachi-2900 UV-Visible spectrophotometer, Tokyo, Japan.

2.8.1. Estimation of amylase:

The reducing sugars liberated by the action of alpha-amylase on starch was estimated by Somogyi-Nelson method using 3,5-dinitrosalicylic acid (DNS) (Bernfeld 1955) using starch (1% W/V) as substrate. One

unit of activity (U) is defined as the amount of enzyme need to produce 1 mg of maltose per min per mL of homogenate at 37 °C. The specific activity is expressed as U mg⁻¹ protein.

2.9. Statistical analysis:

All data were analyzed by SAS (1998). One-way analysis of variance (ANOVA) was used to determine whether significant variation existed between the treatments. When overall differences were found, they were tested by Duncan's multiple rang test as described by Duncan (1955).

3.10. Economical evaluation:

A simple economic analysis was conducted for different experimental treatments to estimate the cost of feed required to produce a unit of fish biomass. The estimation was based on local retail sale market price of all the dietary in gradients at the time of the study. These prices were as follows Table (2).

RESULTS

Water quality in all tanks were observed to be normal and remained within ranges allowing for high growth rate and production of Nile tilapia (Table 3). Water temperature was varied from 26.50 - 27.80 °C, pH ranged from 6.90 to 7.40, ammonia at 0.010 mg/l to 0.018 mg/l and DO range from 6.15 to 6.62 mg/l. The exact mode of action of the probiotic has not been fully elucidated and there is continuous argue about its effect on the water quality Shefat, (2018). In the present study, there is no obvious effect of the probiotics added to feeds on water quality, this agrees with the finding of Yanbo and Zirong (2006) and Essa *et al.*, (2011). and (El-Haroun *et al.*, 2006), they found that, commercial probiotics made from *Bacillus licheniformis* and *B. subtilis* led to optimized dissolved oxygen and ammonia levels.

Table 2. Feed raw materials price table

| The components | Prices/kg |
|---|-----------|
| Fish meal | 70 |
| Gluten | 20 |
| Soybean meal | 17 |
| Wheat bran | 7 |
| Corn | 8 |
| Vit and Min. Mix ¹ | 40 |
| Fish oil | 140 |
| Soybean oil | 90 |
| <i>Bacillus subtilis</i> & <i>B. licheniformis</i> (g /kg) | 200 |
| Manan and frac (g /kg) | 200 |
| Total | 792 |

Growth performances:

The growth performance including IBW, FBW, SGR and survival rate of Nile tilapia are shown in (Table 4). No significant differences (P >0.05) were observed in IBW among treatments. Fish fed the experimental diets T1,

T2, T3 and T4 exhibited higher FBW and SGR compared to the control diet. Nile tilapia fed BLM diet had a significantly ($P \leq 0.05$) highest WG, WG%, SGR than the rest of experimental group. This results were in agreement with (Mugwanya *et al.*, 2021). Hassaan *et al.* (2014) indicated that, *O. niloticus* fed the diet contained (0.48×10^6 CUF g^{-1} *B. licheniformis* and 1.0% yeast extract) gained the highest BW, WG and SGR compared with fish fed control and the diets supplemented with *B. licheniformis* or yeast extract singly. Recently, Hassaan *et al.* (2017) showed that highest values of BW, WG and SGR of *O. niloticus* fed diets supplemented with the two combinations of 5 g malic acid/kg and 1.1×10^5 cfu/g *B. subtilis* and 10 g malic acid/kg and 1.1×10^5 cfu/g *B. subtilis*. Biosynthesis of vitamins by probiotic bacteria has been reviewed by (Gu and Li, 2016), showing that different probiotic species could be involved in different vitamin synthesis pathways. The beneficial effects of *Lactobacillus* sp on growth response have been observed in Nile tilapia by Lara-Flores *et al.* (2003), sea bream, *Sparus aurata* (Suzer *et al.*, 2008) and European sea bass *Dicentrarchus labrax* (Carnevali *et al.*, 2006).

The probiotics supplementation of the experimental diets resulted in higher growth and feed utilization as compared with the control diet. The increase in growth of tilapia by inclusion of *B. subtilis* NIOFSD017 may be due to that most of *Bacillus* spp can produce secondary metabolites which have been used industrially for production of antibiotics, bioinsecticides, fine chemicals and enzymes that readily hydrolyze carbohydrates, lipids and proteins into sugars, fatty acids, peptides and amino acids (Olmos *et al.*, 1998). Similar results were found for common carp, *Cyprinus carpio*

(Yanbo and Zirong 2006), red drum, *Sciaenops ocellatus*, (Li *et al.*, 2005) and Japanese flounder, *Paralichthys. Olivaceus* juveniles (Taoka *et al.*, 2006). Such beneficial effects of yeast have been observed in Nile tilapia and other fish species (Taoka *et al.*, 2006). Whereas *Bacillus* sp. have been successfully used as a probiotic to enhance growth of these fish. (Venkat *et al.*, 2004).

Feed utilization:

Results of Table (5) indicated that, the experimental diets with combination of probiotic and prebiotic significantly increased feed intake (FI) and improved FCR, FE and PER which was subsequently followed by an increase in the growth performance, and this means that the use of probiotics can decrease the amount of feed necessary for animal growth and therefore reduce production costs. The highest PER was recorded for T3 (2.08), while the lowest PER was recorded for control treatment (1.39). This results is in agreement with Youngjin *et al.*, (2019). Also, incorporation of prebiotic in *O. niloticus* diets significantly ($P < 0.05$) increased feed intake and referring to the dietary symbiotic effect results of the present study showed that, dietary symbiotic showed the highest FI, the best FCR, and PER compared to fish groups fed the control diet. All the diets tested reduced the FCR to less than 2, except the control diet and T4. The bioactive attributes of dietary prebiotics and probiotics accelerated a reduction in FCR, which indicates that the tested diets were economically viable. In addition, the inclusion of dietary prebiotics and probiotics increased the protein efficiency ratio, which was a positive result because PER helps to reduce the FCR (USAID 2011).

Table 3: Water quality of Nile tilapia tanks measured during the experiment period

| | Control | (BSM)T1 | (BSF)T2 | (BLM)T3 | (BLF)T4 |
|---------------------------------|--------------|---------------|---------------|--------------|---------------|
| Temperature. (°C) | 26.80 ± 0.20 | 27.20 ± 0.18 | 27.40 ± 0.30 | 26.50 ± 0.11 | 27.80 ± 0.24 |
| PH | 7.20 ± 0.31 | 7.31 ± 0.10 | 6.9 ± 0.14 | 7.12 ± 0.19 | 7.40 ± 0.13 |
| Ammonia mg/L | 0.012 ± 0.01 | 0.018 ± 0.002 | 0.015 ± 0.002 | 0.018 ± 0.01 | 0.010 ± 0.005 |
| Dissolved o ₂ (mg/l) | 6.30 ± 0.03 | 6.23 ± 0.03 | 6.15 ± 0.14 | 6.62 ± 0.44 | 6.31 ± 0.14 |

¹ Values (Mean ± Standard Deviation) in each row with different superscripts are significantly different ($P < 0.05$) BSM- *Bacillus. subtilis* with mannan. BSF- *Bacillus. subtilis* with fructooligosaccharide. BLM- *Bacillus. Licheniformis* with mannan BLF- *Bacillus. Licheniformis* with fructooligosaccharide

Table 4: Growth performance of Nile tilapia fingerlings fed on the experimental diets for 70 days

| Parameters | Control | BSM(T1) | BSF (T2) | BLM (T3) | BLF(T4) |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Initial weight (g) (IBW) | 10.50 ± 0.56 | 10.55 ± 0.23 | 10.45 ± 0.18 | 10.40 ± 0.20 | 10.42 ± 0.45 |
| Final Weight (g) (FBW) | 25.9 ± 0.12 ^d | 29.05 ± 0.14 ^c | 32.45 ± 0.14 ^b | 35.90 ± 0.10 ^a | 25.8 ± 0.10 ^d |
| Weight gain (g) (WG) | 15.40 ± 0.22 ^d | 18.50 ± 0.22 ^c | 22.00 ± 0.20 ^b | 25.50 ± 0.21 ^a | 15.38 ± 0.22 ^d |
| Weight gain % | 146.67 ± 0.10 ^d | 175.35 ± 0.12 ^c | 210.53 ± 0.12 ^b | 245.19 ± 0.13 ^a | 147.60 ± 0.14 ^d |
| Specific Growth Rate (SGR) | 1.29 ± 0.02 ^d | 1.45 ± 0.02 ^c | 1.62 ± 0.23 ^b | 1.76 ± 0.10 ^a | 1.30 ± 0.02 ^d |
| Survival Rat % | 100 ± 0.00 ^a | 94.3 ± 1.56 ^d | 98.7 ± 0.50 ^b | 100 ± 5.34 ^a | 96.7 ± 5.77 ^c |

Values (Mean ± Standard Deviation) in each row with different superscripts are significantly different ($P < 0.05$)

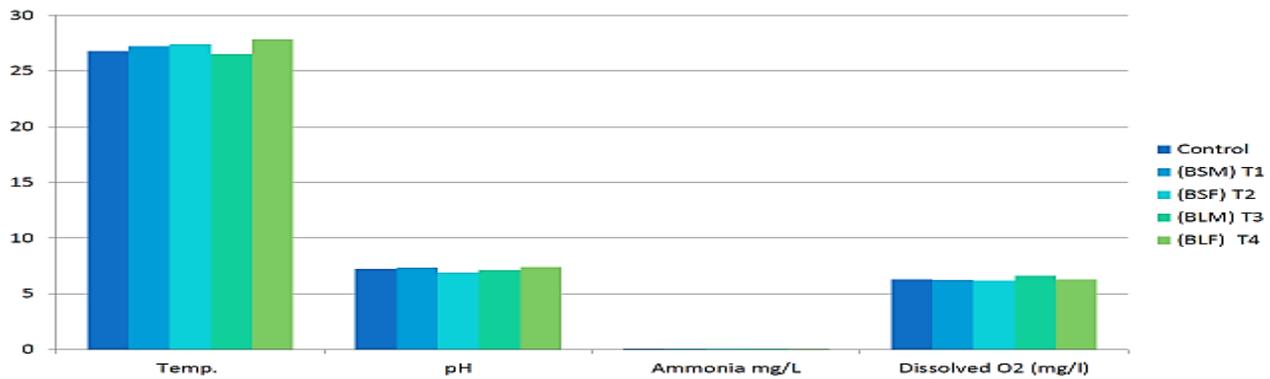


Figure (1): Overall percentage of progressive motility before and after filtration as affected by the type of filters (a, b shows differences between means at $P \leq 0.05$)

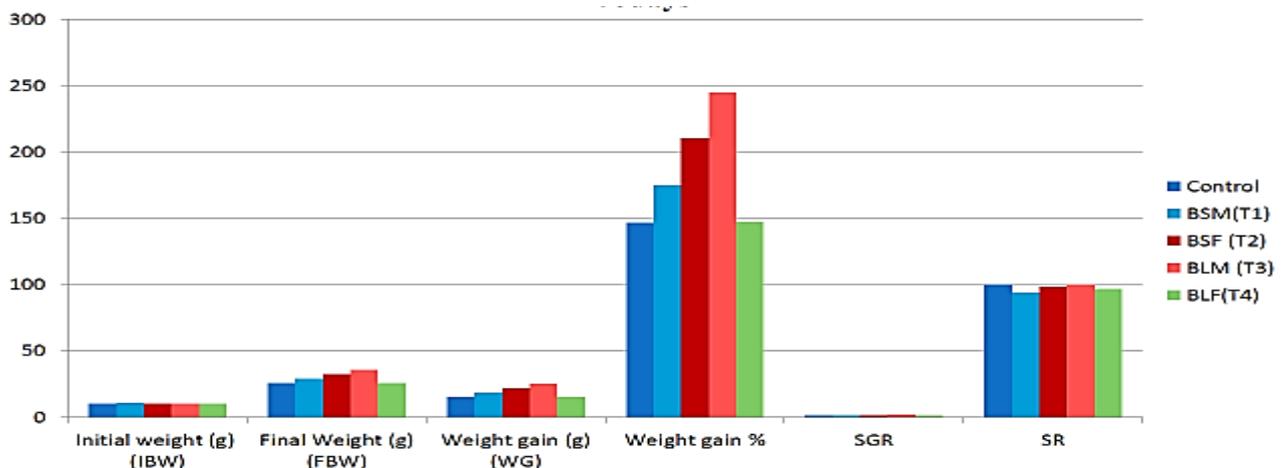


Figure (2): Growth performance of Nile tilapia fingerlings fed on the experimental diets for 70 days

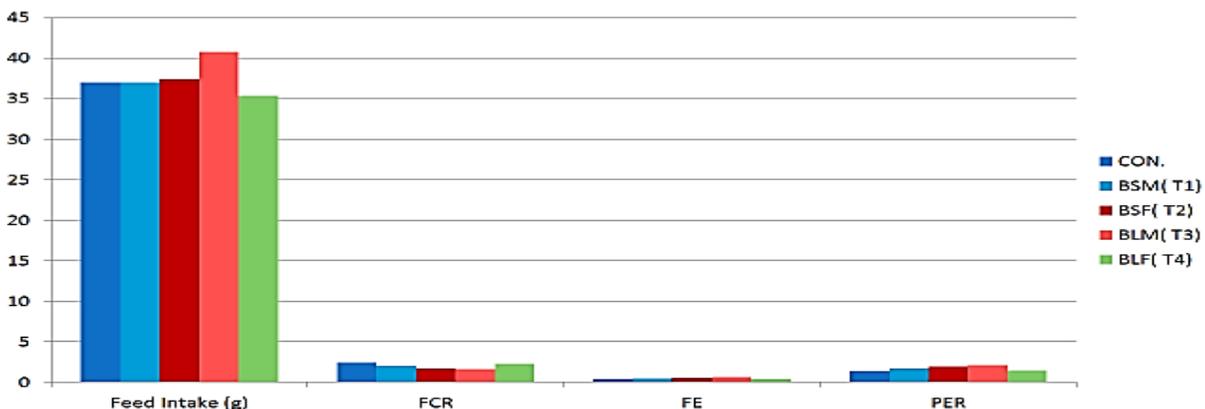


Figure (3): Feed utilization of Nile tilapia (*Oreochromis niloticus*) fingerlings fed on the experimental diets for 70 days

Whole -body proximate composition:

Whole body composition data are presented in (Table 6). The Moisture content showed no significant differences ($P > 0.05$) among fish fed the experimental diets and it is ranged from 74.20 to 75.30%. Significantly, the uppermost two values (61.30 and 61.60%) of crude protein were achieved for fish fed diets T2 and T3 with no significant difference ($P > 0.05$). Average fat content of fish fed T1, to T3 and the control were $23.10 \pm 0.21\%$ to $21.22 \pm 0.41\%$. According to Hephher (1990), endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding

frequency, temperature etc.) affect the body composition of fish.

Enzyme Activity:

The total (U ml^{-1}) and specific (U mg protein^{-1}) amylase activities of Nile tilapia in the present study fed different dietary probiotics and prebiotics were significantly higher ($P \leq 0.05$) than the control diet (Table7). The highest total amylase activities were recorded for fish fed either T2 or T3 respectively (42.60 and 40.80 U ml^{-1}) and the lowest significant values ($P \leq 0.05$) were recorded for the control diet. The same trend was observed for specific amylase activities.

Table 5: Feed utilization of Nile tilapia (*Oreochromis niloticus*) fingerlings fed on the experimental diets for 70 days

| Parameters | CON. | BSM(T1) | BSF(T2) | BLM(T3) | BLF(T4) |
|------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Feed Intake (g) | 36.96± 1.70 ^c | 37.00± 1.23 ^b | 37.40± 1.29 ^b | 40.80± 1.80 ^a | 35.37± 1.21 ^d |
| FCR ¹ | 2.40± 0.77 ^a | 2.0± 0.34 ^c | 1.7± 0.017 ^d | 1.60± 0.06 ^d | 2.30± 0.21 ^a |
| FE ² | 0.42± 1.60 ^d | 0.50± 0.01 ^c | 0.59± 0.08 ^b | 0.63± 0.93 ^a | 0.43± 0.003 ^d |
| PER ³ | 1.39±0.80 ^c | 1.67± 0.04 ^b | 1.96± 0.002 ^a | 2.08± 0.15 ^a | 1.45± 0.04 ^d |

Values (Mean ± Standard Deviation) in each row with different superscripts are significantly different ($P < 0.05$) 1- feed conversion ratio 2- Feed Efficiency Protein 3- Efficiency Ratio.

Table 6: Body composition Nile tilapia (*Oreochromis niloticus*) fingerlings fed the experimental diets for 70 days.

| Item | Diets | | | | | |
|---------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Initial | Control | T1(BSM) | T2(BSF) | T3(BLM) | T4(BLF) |
| Moisture | 74.18±0.23 | 74.30±0.23 | 75.30±0.20 ^a | 74.30±0.21 | 74.30±0.22 | 74.30±0.23 |
| Crude protein | 59.18±0.23 ^c | 60.30±0.23 ^b | 60.20±0.20 ^b | 61.30±0.21 ^a | 61.60±0.22 ^a | 60.90±0.23 ^b |
| Crude fat | 21.50±0.23 ^c | 22.10±0.23 ^b | 23.10±0.21 ^a | 21.60±0.22 ^c | 21.22±0.41 ^c | 21.60±0.21 ^c |
| Crude ash | 16.70±0.21 ^a | 15.20±0.20 ^b | 15.30±0.23 ^b | 15.10±0.23 ^b | 15.30±0.22 ^b | 15.10±0.22 ^b |

¹ Values (Mean ± Standard Deviation) in the same row with different superscript are significantly different ($P < 0.05$) b shows differences between means at $P \leq 0.05$

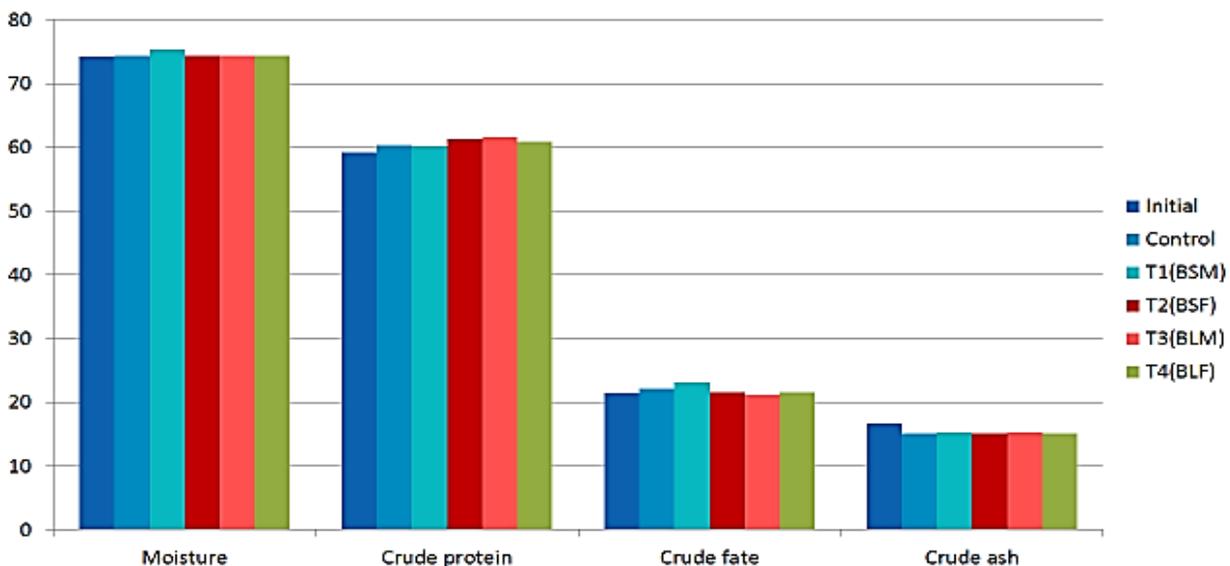


Figure 4: Chemical composition % of Nile tilapia (*O. niloticus*) fed on experimental diet on (DM basis)

Hematological parameters:

The blood content can be a good indicator of fish health status. Changes in different blood parameters have been reported in response to the immunostimulant compound (Jafarzadeh *et al.*, 2015 and Talpur and Ikhwanuddin, 2012). The results of the present study showed that most hematological parameters in fish fed with supplemented diets significantly changed in

comparison with the fish fed with the basal diet (Table 8). The results indicated that there was a significant difference among fish fed the experimental diets ($P > .05$). These results in were agreement with Youngjin *et al.*, (2019). Antibiotics can be substituted with synbiotics to increase antioxidant activity and blood biochemical markers (El-Nobi, *et al.*, 2021).

Table 7: Amylase enzyme activity of Nile tilapia (*O. niloticus*) fingerlings fed the experimental diets for 70 days

| Treatments | Protein content Mg/ml | Enzyme activity | |
|------------|--------------------------|---------------------------|----------------------------------|
| | | Total (unit/ml) | Specific activity (mg p/protein) |
| Control | 12.40±0.60 | 30.20 ^d ±1.31 | 2.40 ^d ±0.13 |
| T1 BSM | 12.10±0.55 | 35.20 ^c ±3.90 | 2.80 ^c ±0.28 |
| T2 BSF | 12.01±0.55 | 42.60 ^a ±2.32 | 3.50 ^a ±0.31 |
| T3 BLM | 12.30±0.42 | 40.80 ^{ab} ±2.90 | 3.30 ^b ±0.20 |
| T4 BLF | 12.12±0.70 | 38.50 ^b ±3.80 | 3.30 ^b ±0.30 |

¹ Values (Mean ± Standard Deviation) in the same with different superscript are significantly different ($P < 0.05$)

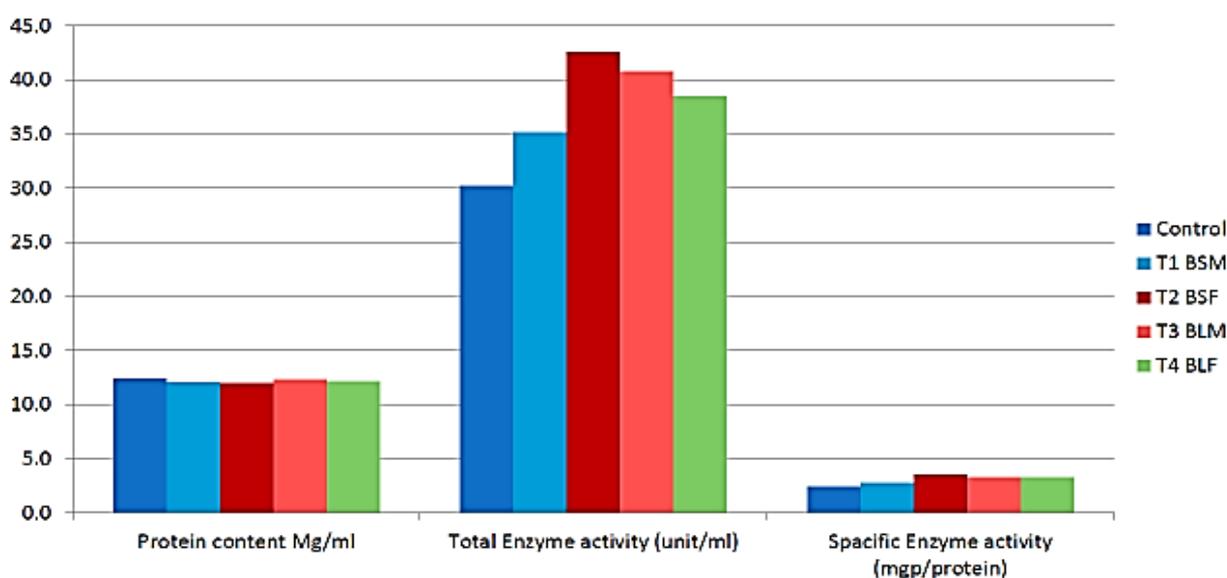


Figure 5. Amylase enzyme activity of Nile tilapia (*O. niloticus*) fingerlings fed the experimental diets for 70 days

Table 8: Hematological parameters of Nile tilapia I fed the experimental diets for 70 days

| Items | Diets | | | | | |
|---|---------------------------|--------------------------|---------------------------|---------------------------|--|--------------------------|
| | Cont. | BSM T1 | BSF T2 | BLM T3 | | BLF T4 |
| Hb (g/dl) | 10.51± 130 ^b | 10.21± 1.70 ^a | 11.43± 1.13 ^c | 11.32± 1.90 ^a | | 10.82± 1.23 ^c |
| RBC (10 ⁶)/cmm | 1.82 ± 0.030 ^d | 1.88± 0.004 ^c | 1.91± 0.05 ^b | 1.97 ± 1.70 ^a | | 1.85 ± 0.10 ^c |
| WBC(10 ³) | 36.83± 1.70 ^c | 36.11± 1.67 ^c | 37.30± 1.80 ^b | 38.36 ± 1.09 ^a | | 37.80± 1.19 ^b |
| AST U/mL ¹ | 27.00 ± 22.3 ^a | 23.7 ± 21.0 ^c | 20.30 ± 4.62 ^d | 18.3 ± 12.7 ^c | | 25.0 ± 25.2 ^b |
| ALT U/mL | 28.00±0.58 ^a | 24.67±1.20 ^c | 22.00±1.15 ^d | 21.00±0.58 ^c | | 26.00±0.58 ^b |
| TP (g dL ⁻¹) ² | 6.30 ± 0.47 ^a | 5.00 ± 0.10 ^b | 5.01 ± 0.32 ^b | 4.40 ± 0.32 ^c | | 5.20± 0.15 ^b |
| GLU (mg dL ⁻¹) ³ | 105 ± 9.2 ^a | 82.5 ± 19.1 ^c | 71.0 ± 6.21 ^d | 82.3 ± 13.5 ^c | | 96.0 ± 15.4 ^b |

• Values are mean ± SD from three replicate groups of fish where the values in each row with different superscripts are significantly different ($P < .05$).

• Diets represent; CON = basal diet, BSM = *B. subtilis* with MOS in CON, BSF = *B. subtilis* with FOS in CON, BLM = *B. licheniformis* with MOS in CON and BLF = *B. licheniformis* with FOS in CON.

• ¹ GOT (U/L): Glutamic pyruvic transaminase.

• ² TP (g/dl): Total protein.

• ³ GLU (mg/dl): Glucose.

Organosomatic indices:

Table (9) shows the *organosomatic* indices of Nile tilapia fingerlings fed the experimental diets for 70 days. HSI of fish fed *BSF* and *BLM* diets was significantly higher ($P \leq 0.05$) than those of fish fed CON diet ($p \leq .05$). However, there were no significant differences among fish fed BSM, BSF, BLM and BLF

diets ($p > 0.05$). Also, there were significant differences in VSI among fish fed the experimental diets ($p > 0.05$). In agreement with Park *et al.*, (2019). The present study also revealed that the inclusion of dietary.

4.9. Economic evaluation:

Calculations of economic efficiency of the tested diets based on the cost of feed, costs of one Kg gain in weight and its ratio with the control group are shown in Table (10). Feed costs and costs per kg gain (L.E) were the highest for the T3 diet (7.46 L.E) and gradually decreased with the type of feed additives (probiotic and prebiotic). The lowest relative percentage of feed cost/ kg fish being to be 83.92, 71.72, 68.35 and 96.11 for diets T1, T2, T3, T4 respectively. Moreover, the relative percentage of feed cost/ kg gain was found

to be 99.90, 100.0, 89.0, 94.97, and 86.22, (L.E) for diets control, T1, T2, T3 and T4 respectively. These results indicate that dietary prebiotics and probiotics improved growth and feed utilization parameters of Nile tilapia fingerlings. On the other hand, the incorporation of probiotic and prebiotic in Nile tilapia fingerlings diets seemed to be economic. The drop in feed costs was clearly seen for the feed cost/Kg weight gain, which fell when probiotic Nile tilapia fingerling diet levels were increased, in accordance with Khattab *et al.* (2004) and Mohamed. (2007).

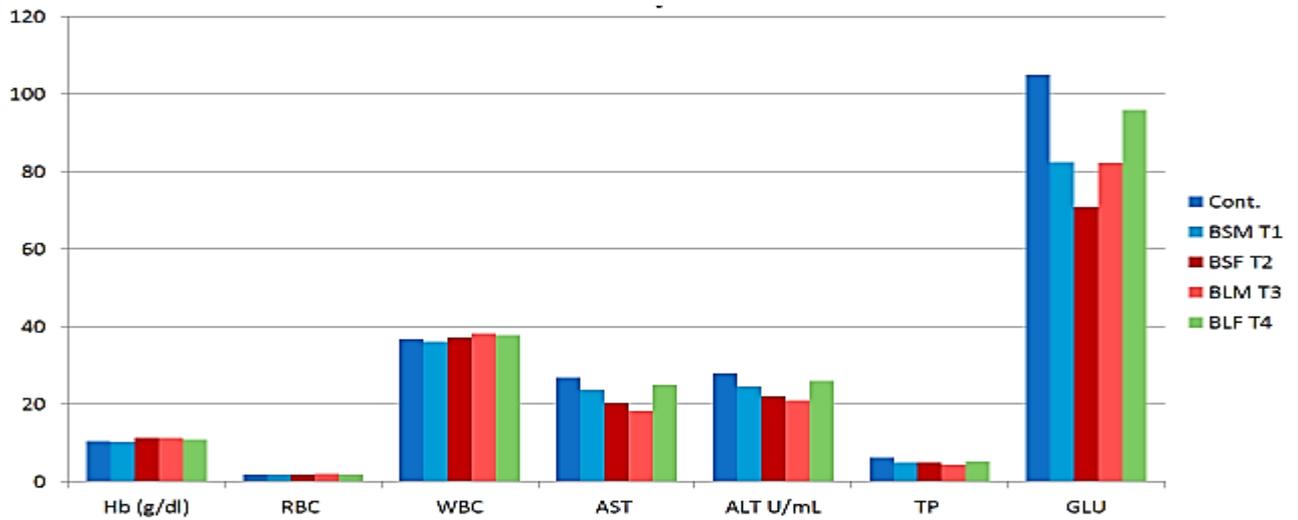


Figure 6: Hematological parameters of Nile tilapia l fed the experimental diets for 70 days

Table (9): Organosomatic indices of Nile tilapia (*Oreochromis niloticus*) fingerlings fed the experimental diets for 70 days.

| Prameters | CON | T1 (BSM) | T2 (BSF) | T3 (BLM) | T4 (BLF) |
|------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|
| HSI ¹ | 1.07 ± 0.22 ^b | 1.60 ± 0.48 ^a | 1.50 ± 0.39 ^a | 1.47 ± 0.30 ^{ab} | 1.44 ± 0.21 ^{ab} |
| VSI ² | 1.41 ± 0.50 ^c | 1.93 ± 0.60 ^a | 1.95 ± 0.86 ^a | 1.86 ± 0.65 ^b | 1.84 ± 0.55 ^b |

Values are mean ± SD from nine replicate groups of fish where the values in each row with different superscripts are significantly different (p < .05).

- 1 Hematosomatic index (HSI, %) = (liver weight/ body weight) × 100.
- 2 Visceralsomatic index (VSI, %) = (viscera weight/ body weight) × 100.

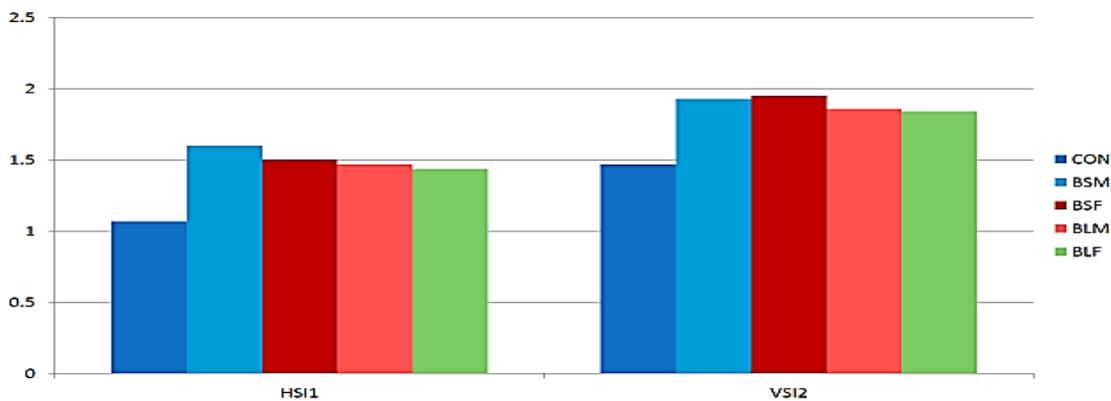


Figure 7: Organosomatic indices of Nile tilapia fingerlings fed the experimental diets for 70 days

Table 10: Economical Evaluation of Experimental diets for fingerlings Nile Tilapia

| Parameters | Treatment | | | | |
|---|-----------|----------|----------|----------|----------|
| | Cont. | T1 (BSM) | T2 (BSF) | T3 (BLM) | T4 (BLF) |
| Cost /kg diet (LE) ¹ | 7.28 | 7.33 | 7.37 | 7.46 | 7.30 |
| Consumed feed to produce 1kg fish (kg) ² | 2.40 | 2.0 | 1.7 | 1.60 | 2.3 |
| Feed cost per kg fresh ³ fish (LE) | 17.47 | 14.66 | 12.53 | 11.94 | 16.79 |
| Relative % of feed cost/ kg fish ⁴ | 100 | 83.92 | 71.72 | 68.35 | 96.11 |
| Feed cost /1Kg gain (LE) ⁵ | 10.73 | 10.74 | 9.56 | 10.20 | 9.26 |
| Relative % of feed cost of Kg gain ⁶ | 99.9 | 100 | 89.0 | 94.97 | 86.22 |

- 1- Cost per Kg diet L.E.
 - 2- Feed intake per fish per period/ final weight per fish Kg/Kg
 - 3- Step1x step2
 - 4- Respective figures for step 3/ highest figure in this step
 - 5- Feed intake per Kg gain x step1
 - 6- Respective figures for step 5/ highest figure in this step
- Egypt Feed Ingredients Price at start of 2019.

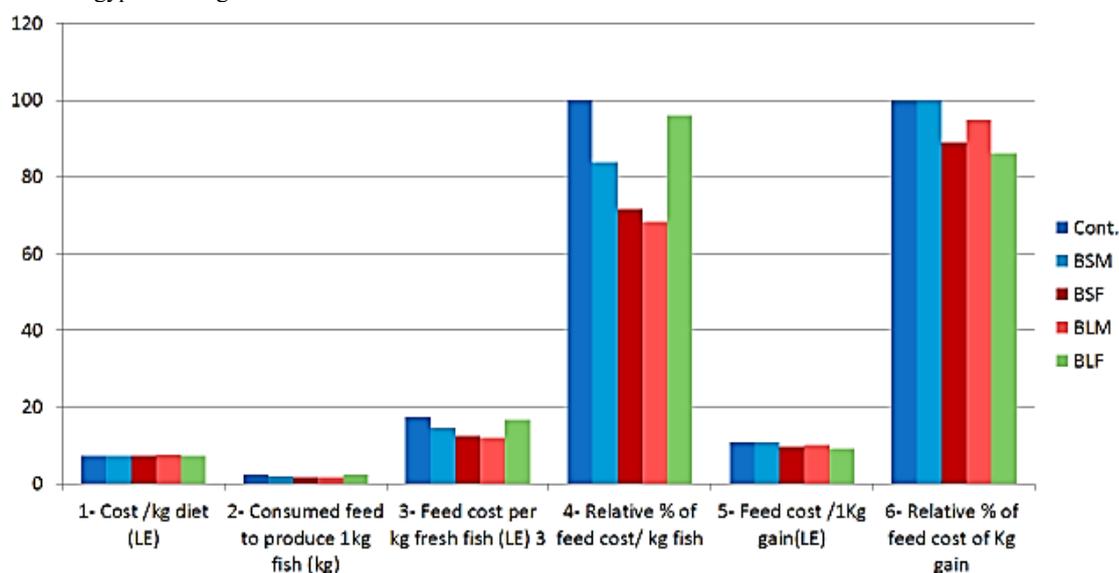


Figure 8: Economical Evaluation of Experimental diets for fingerlings Nile Tilapia

CONCLUSION

In conclusion, dietary *B. subtilis* with fructo-oligosaccharide (BSF) and *B. licheniformis* with mannan oligosaccharide (BLM) could have beneficial effects on growth performance, feed utilization and economical study in Nile tilapia (*Oreochromis niloticus*) fingerlings. These results indicate that specific combinations of pro- and prebiotics are important in exerting beneficial effects. Further study on determining the optimum dose of those combinations might help to improve Nile tilapia (*Oreochromis niloticus*) culture.

REFERENCES

- Alarcón, F.J.; Moyano, F.J. and Díaz, M. (1999). Effect of inhibitors present in protein sources on digestive proteases of juvenile sea bream (*Sparus aurata*). *Aquat. Living Resour.*, 12, 233– 238.
- Abdel-Aziz, M. H.; Zoromba, M. S.; Bassyouni, M.; Zwawi, M.; Alshehri, A. A. and Al-Hossainy, A. F. (2020). Synthesis and characterization of Co-Al mixed oxide nanoparticles via thermal decomposition route of layered double hydroxide. *Journal of Molecular Structure*, 1206, 127679.
- AOAC (2019). Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, 17th edition. Washington DC, USA. 771pp.
- APHA (2005). Standard methods for the examination of water and waste- water, 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC
- Bai, J. (1997). Estimation of a change point in multiple regression models. *Review of Economics and Statistics*, 79(4), 551-563.
- Bernfeld, P. (1955). Amylases, alpha and beta. In: *Method. Enzymol I* (P. Bernfeld, S.P. Colowick and N.O. Kaplan eds), pp. 149– 158. Academic Press Inc., New York. *Biotechnology* 6, 97-103.

- Boyd, C. E. and Massaut, L. (1999). Risks associated with the use of chemicals in pond aquaculture. *Aquacultural engineering*, 20(2), 113-132.
- Carnevali, O.; De Vivo, L.; Sulpizio, R.; Gioacchini, G.; Olivotto, I.; Silvi, S. And Cresci, A. (2006). Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture* 258, 430-438
- Duncan D.B. (1955). Multiple range and Multiple F test *Biometrice* 11:1-42.
- El-Haroun, E. R.; Goda, A. S. and Kabir Chowdhury, M. A. (2006). Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquaculture Research*, 37(14), 1473-1480.
- El-Nobi, G.; Hassanin, M.; Khalil, A.; Mohammed, A.; Amer, S.; Montaser, M. and El-Sharnouby, M. Synbiotic. (2021). Effects of *Saccharomyces cerevisiae*, Mannan Oligosaccharides, and β -Glucan on Innate Immunity, Antioxidant Status, and Disease Resistance of Nile Tilapia, *Oreochromis niloticus*. *Antibiotics* 2021, 10, 567
- El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., and Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. *Fish & Shellfish Immunology*, 117, 36-52.
- Elsabagh, M.; Mohamed, R.; Moustafa, E. M.; Hamza, A.; Farrag, F.; Decamp, O.; Dawood, M. A. and M. Eltholth. (2018). "Assessing the impact of Bacillus strains mixture probiotic on water quality, growth Page 20/27 performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*." *Aquaculture nutrition* 24(6): 1613-1622
- Essa, S.; Rabanel, J. M. and Hildgen, P. (2011). Characterization of rhodamine loaded PEG-g-PLA nanoparticles (NPs): effect of poly (ethylene glycol) grafting density. *International journal of pharmaceuticals*, 411(1-2), 178-187.
- GAFRD, (2018). General Authority for Fish Resources Development. Statistical Report on Fish Production. Cairo, Egypt.
- Gu, J.; Lu, Z.; Li, H. and Li, V. O. (2016). Incorporating copying mechanism in sequence-to-sequence learning. arXiv preprint arXiv:1603.06393.
- Hassaan, M.S.; Soltan, M.A. and Ghonemy, M.M.R. (2014). Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). *Egypt. J. Aquat. Res.* 2014, 40, 199-208
- Hassaan, M.S.; Soltan, M.A.; Jarmolowicz, S. and Abdo, H.S. (2017). Combined effects of dietary malic acid and *Bacillus subtilis* on growth, gut microbiota and blood parameters of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* · February 2017. 1- 11.
- Hassaan, M.S.; Soltan, M.A. and Ghonemy, M.M.R. (2014). Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). *Egypt. J. Aquat. Res.* 2014, 40, 199-208
- Hassaan, M.S.; Soltan, M.A.; Jarmolowicz, S. and Abdo, H.S. (2017). Combined effects of dietary malic acid and *Bacillus subtilis* on growth, gut microbiota and blood parameters of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* · February 2017. 1- 11.
- Jafarzadeh. E.; Khara, H. and Ahmadnezhad, M. (2015). Effects of synbiotic (Biomin IMBO) on haematological and immunological components of Russian sturgeon, *Acipenser guldenstadti*. *Comparative Clinical Pathology* 24:1317-1323. doi: 10.1007/s00580-015-2071-6.
- Jobling M (1983). A short review and critique of methodology used in fish growth and nutrition studies. *J Fish Biol* 23:685-703
- Khattab, Y. A. E.; Shalaby, A. M. E.; Sharaf Saffa, M., El-Marakby, H. and RizlAlla, E. H. (2004). The physiological changes and growth performance of the Nile Tilapia *Oreochromis niloticus* after feeding with Biogen® as growth promoter. *Egypt. J. Aquat. Biol. And Fish.* Vol. 8, No.2: 145-158.
- Kumar, A.; Roberts, D.; Wood, K. E.; Light, B.; Parrillo, J. E.; Sharma, S. and Cheang, M. (2006). Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical care medicine*, 34(6), 1589-1596.
- Lara-Flores, M.; Olvera-Novoa, M. A.; Guzmán-Méndez, B. E. and López-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216(1-4), 193-201.
- Li, P.; Delbert, M. and Gatlin, III. (2005). Evaluation of the prebiotic GroBiotic®-A and brewer's yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops* × *M. saxatilis*) challenged in situ with *Mycobacterium marinum*. *Aquaculture journal*, Volume 248, Issues 1-4, 29 July 2005, Pages 197-205.
- Lowry, O. H.; Rosebrough, N.J.; Lewis .F,A. and Randall, R.J. (1951). Protein measurement with the folin Phenol Reagent, *Journal of Biological Chemistry* 193(1):256-275
- Mohamed, K. A. (2007). Effect of Using Probiotic and Yeast as Growth Promoters in Commercial Diet of Tilapia (*Oreochromis niloticus*) Fingerlings. *Agricultural Research Journal*, Suez Canal University, 7 (3): 41-47.
- Mugwanya, M.; Dawood, M. A.; Kimera, F. and Sewilam, H. (2021). Updating the role of probiotics, prebiotics, and synbiotics for Tilapia aquaculture as leading candidates for food sustainability: A review *Probiotics and Antimicrobial Proteins* (2021), pp. 1-28.

- Olmos, S.J.; Sanchez, G.A. and DeAnda, R. (1998). Regulations of the *apre* (subtilisin) gen in *abr B* mutants of *Bacillus subtilis* Asia pacific journal of molecular biology and biotechnology 6, 97-103
- Panigrahi, A.; Kiron, V.; Puangkaew, J.; Kobayashi, T.; Satoh, S. and Sugita, H. (2005). The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, 243(1-4), 241-254.
- Park, T.; Liu, M. Y.; Wang, T. C. and Zhu, J. Y. (2019). Semantic image synthesis with spatially-adaptive normalization. In Proceedings of the IEEE/CVF conference on computer vision and pattern recognition (pp. 2337-2346).
- Reitman, S., and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, 28(1), 56-63.
- Ringø, E.; Hoseinifar, S.H.; Ghosh, K.; van Doan, H.; Beck, B.R. and Song, S.K. (2018). Lactic acid bacteria 2080 in finfish – an update. *Front. Microbiol.* 9: 1818. doi: 10.3389/fmicb.2018.01818
- SAS. Statistical Analysis System, (1998). SAS program Ver. 6. 12, SAS institute incorporation. Cary. NC 27513 USA.
- Shefat, S. H. T. (2018). Probiotic strains used in aquaculture. *International Research Journal of Microbiology*, 7(2), 43-55.
- Suzer, C.; Çoban, D.; Kamaci, H. O.; Saka, S.; Firat, K.; Ogtucuoglu, O. and Küçüksari, H. (2008). *Lactobacillus* spp. Bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) Larvae: Effects on growth performance and digestive enzyme activities. *Aquaculture* 280, 140-145.
- Svobodová Z.; Pravda, D. and Paláckova, J. (1991). Unified methods of haematological examination of fish. *Vodnany: Research Institute of Fish Culture and Hydrobiology. Methods No. 20*
- Talpur, A. D. and Ikhwanuddin, M. H. D. (2012). Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture*, 364, 6– 12.
- Taoka, Y.; Maeda, H.; Jo, J.Y.; Jeon, M.J.; Bai, S.C.; Lee, W.J. and Yuge, Koshio, S. K. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. *Fisheries Science* 72, 310- 321
- United States Agency for International Development (USAID). (2011). Technical Bulletin# 07. Phnom Penh, Cambodia: USAID; 2011.
- Venkat, H.K.; Narottam, P.S. and Jain, K.K. (2004). Effect of feeding *Lactobacillus* based- probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* Man). *Aquaculture Research* 35,501-507
- Verschuere, L.; Rombaut, G.; Sorgeloos, P. and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and molecular biology reviews*, 64(4), 655-671.
- Xia, Y. E.M.; Yu, M. and Lu, J. X. (2020). Effects of dietary probiotic supplementation on the growth, gut health and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus*) *Animal nutrition*, 6(1): 69–79.
- Yanbo, W. And Zirong X. (2006). Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. *Animal feed science and technology* 127, 283-292
- Youngjin, P.; Haham, K.; Seonghun, W.; Ali, H. and Tawheed H. M.d. (2019). Effects of two dietary probiotics (*Bacillus subtilis* or *Licheniformis*) with two prebiotics (mannan or fructo oligosaccharide) in Japanese eel, *Anguilla japonica* *Aquaculture nutrition* Volume 26, (2): 211-600
- Yu, L.; Zhai, Q.; Zhu, J.; Zhang, C.; Li, T.; Liu, X.; Zhao, J.; Zhang, H.; Tian, F. and W. Chen (2017). Dietary *Lactobacillus plantarum* supplementation enhances growth performance and alleviates aluminum toxicity in tilapia *Ecotoxicol. Environ. Saf.*, 143, pp. 307-314,
- Yuan, Y.; Dai, Z.; Zhang, Y. and Gong, Y. Y (2020). Technical efficiency of different farm sizes for tilapia farming in China. *Aquac. Res.*, 51 (1), pp. 307-315.

تأثير أنواع مختلفة من البروبيوتيك والبريبايوتك الغذائي على النمو ، واستخدام العلف ، وأنشطة إنزيمات الجهاز الهضمي للبطني النيليني (اوريوكروماس نيلوتيكس)

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أجريت تجربة تغذية لمدة 70 يوماً للتحقيق في آثار اثنين من البروبيوتيك الغذائي *Bacillus subtilis* KCTC 2217 أو *Bacillus licheniformis* KCCM 11775 مع اثنين من البريبايوتكس، منان قليل السكر (MOS) أو فركتوليغوساكاريد (FOS) على إصبعيات البطني النيليني. وزعت الأسماك التي يبلغ متوسط وزنها 10.8 ± 0.50 جم (متوسط \pm SD) بشكل عشوائي إلى خمس معاملات مع خزانات ثلاثية. نظام غذائي للتحكم الأساسي (CON) وأربعة أنظمة غذائية متزامنة تكمل (*B. subtilis* + MOS (BSM)، *B. subtilis* + FOS (BSF)، *B. licheniformis* + MOS (BLM) و (*B. licheniformis* + FOS (BLF)). أظهرت النتائج ذلك تقدم بكتيريا *B. subtilis* الغذائية مع (FOS (BSF) و (*B. licheniformis* مع (MOS (BLM) أفضل زيادة في الوزن ومعدل نمو محدد عن العلاجات الأخرى. بشكل عام، كانت زيادة الوزن ومعدل النمو النوعي للأسماك التي تم تغذيتها على جميع الأنظمة الغذائية المتزامنة أعلى من علف الأسماك الضابطة. يمكن أن نستنتج أن البروسيلار الرقيقة الغذائية مع (FOS (BSF) و (*B. licheniformis* مع (MOS (BLM) يمكن أن يكون لها آثار مفيدة على أداء النمو واستخدام العلف في إصبعيات البطني النيليني (*Oreochromis niloticus*). توصي النتائج الحالية بدمج البروبيوتيك في علف البطني النيليني كمكملات لتحفيز نمو الأسماك وعضمها.