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Effect of *Bacillus subtilis* and *Chlorella Vulgaris* Supplementation in Diets on Growth Performance, Hematological and Biochemical Variables of Nile Tilapia (*Oreochromis niloticus*) Fingerlings.

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Abstract:

The main objective of this study was to evaluate the possible synergistic effects of *Bacillus subtilis* (Bs) and *Chlorella vulgaris* (Cv) on growth performance, feed utilization, hematological and biochemical variables and immune response of Nile tilapia (*Oreochromis niloticus*) monosex fingerlings. 210 *O. niloticus* fingerlings (with an average weight of 3.55 g placed in 21 aquarium/triplicates for each treatment) were divided into six test diets enriched with Bs (3 or 6 g per kg diet), Cv (2 and 3 g per kg diet) or both Bs and Cv for a period of 14 weeks, in addition to a control diet. The data showed that fish fed Bs or/and Cv had higher final body weights, weight gains, and specific growth rates than the control group, according to the data; the fish fed diets containing Bs and Cv had the highest growth rates. Fish fed Bs and/or Cv had lower feed conversion ratios. Moreover, fish fed both Bs and CV had the lowest ratio. The hematological and biochemical variables showed significant differences between the groups with normal values. The above demonstrates the importance of feeding (*O. niloticus*) monosex diets rich in *B. subtilis* and *C. vulgaris* to improve growth performance and general health.

INTRODUCTION

As one of the primary sources of reasonable animal protein needed for human nourishment, aquaculture has seen a rise in demand recently **(FAO, 2018)**. Therefore, intensive fish farming (which refers to optimizing the yield per unit area) is required to boost fish output **(Dawood *et al.*, 2020a; Herrera *et al.*, 2019)**. However, a number of issues pertaining to environmental stresses have surfaced as a result of fish's limited biological capacity and the availability of natural resources in the aquaculture ecosystem **(De Punder and Pruimboom, 2015)**. Typically, immunological deficiencies brought on by high densities, poor water quality, and erratic weather patterns can cause fish to become infected with outbreaks **(Bonga, 1997; Abdel-Latif *et al.*, 2020a)**.Antibiotics are the primary treatment for infectious infections; nevertheless, repeated chemotherapeutic usage has numerous negative effects on fish health, human health and ecology **(Akanmu, 2018; Dawood and Koshio, 2016)**. As a result, the use of antibiotics in the livestock, poultry and fish production industries has been outlawed in several nations **(Santos and Ramos, 2018)**. There is a growing need for natural substitutes that can lessen antibiotic usage without endangering aquatic life **(Cao *et al*., 2020)**. Fish can be raised with the alternative chemicals by include them in their diets for the duration of the rearing season **(Dawood *et al*., 2019a)**. Fish diets have seen success with a wide range of food additions, such as medicinal herbs and probiotics **(Abdel-Latif *et al*., 2020b; Cao *et al*., 2020; Kothari *et al*., 2019)**. Probiotics are a class of bacteria that enhance the intestinal diversity, capacity for absorption and digestion, and local intestinal immunity within the fish's digestive system **(Pérez-Sánchez *et al*., 2014).** One of the most significant probiotic species employed in many fish species is Bacillus bacteria, whose spores are simple to add to aquafeed and have a reasonably long shelf life **(Ringo *et al*., 2018; Yılmaz *et al*., 2020)**. The advantages of Bacillus species in Nile tilapia (*O. niloticus*) include improved gastrointestinal tract digestion capacity, increased resistance against environmental stressors and outbreaks, and improvements in humoral and adaptive immune responses and local intestinal immunity **(Chantharasophon *et al*., 2011; Han *et al*., 2015; Telli *et al*., 2014)**. Medicinal herbs and their extracts can also be used as food additives to enhance the growth and general health of aquatic animals **(Dawood *et al*., 2018; Van Hai, 2015)**.

A genus of green single-celled algae in the phylum *Chlorophyta* is called *Chlorella*. It lacks flagella and has a spherical form with a diameter of around 2 to 10 μm. The green photosynthetic pigments chlorophyll-a and -b are found in chloroplasts of chlorella **(Becker, 2007)**. It reproduces quickly by photosynthesis, using only carbon dioxide, water, sunlight, and a tiny amount of minerals **(Becker, 2007)**. Because of their chemical makeup, *chlorella spp*. are highly valuable economically. They are also rich in a variety of vital nutrients, including proteins **(Becker, 2007)**, carotenoids **(Del Campo *et al*., 2007)**, and polysaccharides **(Juneja *et al*., 2013)**. The crude protein concentration, which ranges from roughly 15% to 88% of dry matter, has special potential for use as animal feed or for human consumption **(Guccione *et al*., 2014)**. Because of its great demand, quick growth, and affordable pricing, Nile tilapia is the second most important aquatic animal in the world **(El Asely *et al*., 2020a, b)**. When feeding fish, it is often possible to use only one kind of food additive; nevertheless, it appears that a combination of different types works better **(Dawood *et al*., 2015; Yilmaz *et al*., 2019; Yılmaz *et al*., 2020)**. Thus, ensuring the efficacy of adding Bacillus and chlorella as food additives to tilapia diets is the primary Aim of this study.

MATERIALS AND METHODS:

Nile tilapia, *O. niloticus monosex* fingerlings, weighed an average of 3.55 g at the beginning of this experiment. purchased from a private hatchery in the governorate of Kafr El-Sheikh. *O. niloticus* fingerlings were transferred to the Nutrition Laboratory of the Aquaculture Research Unit in Sakha, Kafr El-Sheikh Governorate. The fish were kept in a 1000-liter fiberglass tank to aid in their acclimation to the lab setting. Twenty-one aquarium of 30*40*60 cm, or 72 L, were utilized in this experiment. In each Glass aquaria, ten fish were inserted. Duplicate units were randomized to each therapy before the study started. For two weeks, Fish were allowed to acclimate under trial conditions by feeding them on the control diet at 3% of biomass in equal portions at 8:00, 13:00, and 16:00 hours every day.The baseline ratio was considered as the extruded pelleted diet with a basal formulation (32% crude protein) (Table 1). Control and six test diets (from D1 to D7) were supplemented with Bacillus subtilis and Chlorella vulgaris. The biotechnology Microalgae Culture Unit of the National Research Center (NRC), Giza, Egypt. The algae's proximate analysis consisted of crude protein (45%), total lipids (20%), carbohydrates (20%), crude fibers (5%), and ash 10%. The amounts of Cv examined in this study were shown to be within the sufficient range (3 or 6 g kg diet). A widely accessible commercial product was employed in this investigation. The probiotic $2.5x10^{11}$ CFU/g of *B. subtilis*. In Cairo, Egypt, the National Organization for Drug Control and Research provided the powdered *B. subtilis* strain. As described (**Shelby, *et al*., 2006**). The examined doses of *B. subtilis* were 2 and 3 g per kg diet, as advised by the manufacturer's instructions. Each prepared diet was made by carefully combining all the ingredients. Each kilogram of diet was then combined with 200 milliliters of water, and the resulting concoction of ingredients, feed additive, and water was homogenized to produce a suitable blend of each diet. Every meal was pelleted using a lab pellet machine with a 1 mm diameter die. After that, the wet pellets were left to completely dry at room temperature. The dehydrated pellets were kept in dark plastic containers and chilled at -4°C before being utilized. The proximate analysis of the meals that are being looked at is shown in Table 1. In addition, the chemical content of the produced food samples was evaluated using the **AOAC (2000)** techniques.

Water quality:

very day at 10:00 a.m., the temperature of the water was measured using a mercury thermometer. At eight in the morning, an oxygen meter (YSI model 56; YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) was used to measure the quantity of dissolved oxygen (DO). A DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA) was used to detect total ammonia and nitrite twice a week. In the morning, a pH meter (Orion pH meter, Abilene, Texas, USA) was used to estimate the pH values. For each of the several treatments over the 98-day experimental phase of the study, the water's parameters comprised a temperature of 27.35 ± 0.08°C, a pH of 7.4 ± 0.4, 5.6 ± 0.6 mg/L of dissolved oxygen (DO), less than 0.3 mg/L of total ammonia as nitrogen and nitrite from 0.028 to 0.032 mg/L.For the purpose of raising Nile tilapia, O. niloticus fingerlings, all water quality parameters that were examined (temperature, pH, DO, total ammonia and nitrite) were appropriate and within permissible limits (Boyd, 1990). The robust growth performance is associated with the favorable findings of the water quality criteria, since there was no mortality seen in any of the treatments.

Growth parameters and efficiency of feed:

Every 14 days, measurements of each fish's live body weight (BW/g) and body length (BL/cm) were made in each pond for the duration of the experiment, which lasted 14 weeks. The growth performance parameters were measured using the following formulas:

Condition factor (K). The following formula was used to get the condition factor: -

$K = (W/L^3) \times 100$

Where: W = weight of fish in grams and L = total length of fish in "cm"

Weight gain (WG) = final weight (g) – initial weight (g)

Specific growth rate (SGR):

In order to determine the proximate analysis of the complete fish body, nine fish from each treatment group were preserved at -20°C in plastic bags. The association of Official Analytical Chemists (**AOAC, 2000)** standard operating methods were used to determine the fish body's crude protein, lipid, and ash contents. Crude fiber was estimated according to **Goering and Van Soest (1970)**. Gross energy was calculated according to **NRC (1993)** as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively. To get a consistent weight, the samples were dried in a drying oven (GCA, model 18 EM, Precision Scientific group, Chicago, IL, USA) set to 85 °C for 24 hours. This made it possible to calculate the moisture content.

Blood sampling protocol:

As described **Cicia *et al*. (2012)** Fish were sedated with 100 µg mL−1 MS222 (Tricaine methane-sulfonate, Sigma-Aldrich Co. LLC) prior to blood collection. Two fish each tank (Six fish for each treatment) had their blood samples randomly drawn. Blood samples were drawn from the caudal vein and split into equal parts using sterile 2.5 mL syringes. The first part was stored in a heparinized tube for use in hematological tests, and the second part was refrigerated at 4 °C for three hours after coagulating for thirty minutes at ambient temperature. The clotted samples were then centrifuged for 10 minutes at 4 °C and 3000 rpm in order to obtain serum. After that, the serum was kept at -20 °C until more biochemical, antioxidant, and immunological studies could be conducted.

Blood Hematological assessments:

The **Stoskopf (1993)** technique was followed in order to quantify the erythrocyte and leukocyte counts using a hemocytometer and Natt-Herrik solution. Nonetheless, the hemoglobin level was determined using the cyanmethemoglobin approach, which was endorsed by **Balasubramaniam and Malathi (1992)**. Furthermore, the micro hematocrit method was used to determine the PCV% and calculate the MCV, MCH, and MCHC **(Dacie and Lewis, 1991)**.

Table 1. Formulation and chemical composition of the basal diet (% dry matter).

Ingredients	(control) D1	D2	D3	D4	D5	D6	D7
Fish meal	70	70	70	70	70	70	70
Soybean meal	430	430	430	430	430	430	430
Yellow corn	120	120	120	120	120	120	120
Rice bran	150	150	150	150	150	150	150
Wheat bran	215	213	212	212	209	210	207
Vegetable Oil	5	5	5	5	5	5	5
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium chloride	3	3	3	3	3	3	3
Di calcium	4.5	4.5	4.5	4.5	4.5	4.5	4.5
premix	2	2	2	2	2	2	2
Bacillus subtilis	0	2	3	0	0	2	2
Chlorella vulgaris	0	0	0	3	6	3	6
Sum	1000	1000	1000	1000	1000	1000	1000
Dry matter	90.39	89.93	89.81	90.39	90.40	89.73	89.91
Crud protein	31.68	31.14	31.02	32.63	32.67	31.90	32.39
Ether extract	4.56	4.54	4.66	4.33	4.09	4.91	4.86
Ash	14.49	13.48	13.46	13.56	14.14	15.70	15.06
Fiber	9.25	9.27	8.57	8.80	9.45	8.61	9.13
NFE	41.02	41.58	41.29	39.69	38.66	40.90	39.59
***Gross energy,* (kcal/Kg)**	390.68	389.74	389.00	388.40	382.13	394.73	391.65

1Composition of mineral premix kg-1: manganese,53 g; zinc,40 g; iron, 20 g; copper, 2.7 g; iodine, 0.34 g; selenium, 70 mg; cobalt, 70 mg and calcium carbonate as carrier up to 1 kg. 2Composition of vitamin premix kg-1: vitamin A,8000000 IU; vitamin D3, 2000000 IU; vitamin E, 7000 mg; vitamin K3,1500 mg; vitamin B1, 700 mg; vitamin B2, 3500 mg; vitamin B6, 1000 mg; vitamin B12, 7 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20000 mg; pantothenic acid,7000 mg. 3NFE = 100 – (crude protein + crude lipids+ ash + crude fiber).

In order to calculate the differential leukocytic count, blood smear slides were prepared, allowed to air dry, fixed with methanol for three to five minutes, stained with gimsa stain for eight to ten minutes, rinsed with distilled water, and then allowed to dry at room temperature in accordance with the **Blaxhall and Daisley (1973)** procedure.

Blood biochemical analysis:

The **Wilkinson *et al.* (1972)** technique was followed in measuring the liver function enzymes (AST: aspartate transaminase; ALT: alanine transaminase) using commercial kits (Assay Kit, Biodiagnostic Co., Dokki, Giza, Egypt). In addition, urea and creatinine were tested using commercial kits. The analytical procedures were used on three copies of each treatment.

Digestive enzymes activity:

A wavelength of 580 nm was used for the colorimetric measurement of lipase (REF:281 001 Spectrum. Egyptian business for Biotechnology. Egypt) **(Moss and Henderson, 1999)**. The amylase (CAT. NO. AY 10 50 Biodiagnostic co. Egypt) was measured by colorimetric analysis at 660 nm wave length **(Caraway and Ame, 1959)**

Serum immunity parameters:

The levels of immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG) were measured using commercial kits and AGF Bioscence ELISA Kits. This test uses the competitive inhibition enzyme immunoassay technique. The microtiter plate that comes with this kit has already been precoated with goat anti-rabbit antibody an antibody specific for IgM, standards or samples are added to the relevant microtiter plate wells together with Horseradish Peroxidase (HRP) conjugated IgM. Competitive inhibition occurs when the antibody is applied to both HRP-labeled and unlabeled IgM. Each well's color changes in response to the addition of a substrate solution, which contrasts with the IgM concentration. The color is stopped from developing further and its intensity is measured.

Statistical analysis:

Data analysis was performed using one-way ANOVA for all calculated and estimated data, and Duncan's new multiple range test ((DMRT) was used to confirm any differences between means at a significance level of $P < 0.05$. The findings are displayed as mean ± standard error, and values, all statistical analyses were performed by (SAS, 2012).

RESULTS AND DISCUSSION

Body weight and length:

As stated in Table 2, Fish that were given various treatments had initial body weights ranging from 3.23 to 3.58 g across all treatments. The random distribution of fish around the various experimental treatments is indicated by the negligible differences in beginning body weight among the treatments.

The T6 and T7 treatments released the largest body weights (40.06±0.34 and 40.64±0.41 g, respectively) at the end of this trial, while the T1 (control diet) treatment released the lowest BW (31.01±0.37 g) (Table, 2)

The initial body length varied between 6.04 and 6.38 cm, as this table illustrates, and there were negligible variations between the treatments. By the end of this trial, the treatments T6 and T7 had the highest recorded values of BL (14.17±0.12 and 14.19±0.16 cm), respectively, while the control diet treatment T1 had the lowest BL (12.02±0.12 cm), and there was no significant difference among treatments.

The results of this study (Table, 2) showed that, *O. niloticus* final BW and BL were greatly improved when each of the Bs or/and Cv was added to the baseline diet.

The use of probiotics like Bacillus or Chlorella in tilapia has been the subject of numerous research, but this one is the first to discuss the significance of employing both Bs and CV together on tilapia growth and performance rates. The findings showed that adding both Bs and Cv to tilapia diets had a significant effect on the final body weight indices. The combination of Bs and/or Cv in the diet is linked to accelerating the growth rate of aquatic organisms.

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Table 2. Effect of adding *B. subtilis* and/or *C. vulgaris* levels in fish diet on body weight (g) and length (cm) of *O. niloticus* fingerlings.

Items	Intial weight	Initial length	Final weight	Final length
Control (T1)	3.35±0.27	6.38±0.20	31.01±0.37[e]	12.02±0.12[b]
***B. subtilis* 2 g /Kg (T2)**	3.55±0.17	6.25±0.18	35.13±0.48[c]	12.86±0.11[b]
***B. subtilis* 3 g/Kg (T3)**	3.23±0.20	6.08±0.16	36.99±0.40[b]	12.87±0.12[b]
***C. vulgaris* 3gm/Kg (T4)**	3.49±0.20	6.10±0.18	32.64±0.63[d]	12.95±0.22[b]
***C. vulgaris* 6gm/Kg (T5)**	3.58±0.19	6.16±0.17	37.53±0.45[b]	13.11±0.10[ab]
***B. subtilis* 2 g /Kg + *C. vulgaris* 3gm/Kg (T6)**	3.32±0.20	6.21±0.17	40.06±0.34[a]	14.17±0.12[a]
***B. subtilis* 2 g /Kg + *C. vulgaris* 6gm/Kg (T7)**	3.46±0.17	6.04±0.16	40.64±0.41[a]	14.19±0.16[a]
***P*-Value**	0.8777	0.8391	<0.001	<0.001

± Means followed by the same letter in each column are not significantly different.

Bacillus's function in boosting the diversity of beneficial bacteria in the fish GIT accounts for the crosstalk between algae and Bacillus in the current study's improvement of tilapia development performance **(Dawood *et al.*, 2020b; Elsabagh *et al.*,2018)**. Table 3 results demonstrated that, for fish fed the various experimental diets, the average bod y weight gain over the course of the trial (14 weeks) was 27.65, 31.58, 33.76, 29.16, 33.95, 36.75, and 37.18g, respectively. The WG data showed that, out of all the experimental groups, T7 had the greatest WG value (37.18 g), while the fish group in the control diet (T1) had the lowest WG value (27.65 g).

When compared to the control diet, which was not supplemented with any of the various feed additives utilized in the current investigation, the differences between these means were substantial, demonstrating that all feed additives employed in the experiment considerably improved the live body weight gain of Nile tilapia.

As can be seen in Table 3, the ADG results showed that, of all the experimental groups, T7 had the highest ADG value (0.38 g), while the fish group in the control diet (T1) had the lowest ADG (0.28 g). Additionally, the ADG values for the various experimental diets were significantly higher than those for the control diet Due to their propensity to make food more palatable, Bs/Cv may also play a part in improving feed intake and utilization. Parallel to this work, feeding Nile tilapia with Bacillus sp. improved feed efficiency and growth performance **(Elsabagh *et al.*, 2018; Han *et al.*, 2015)**; **Mahmoud *et al.* (2018)** also observed that feeding spirulina significantly affected the growth rates, the fish fed spirulina supplementation had the highest growth compared to the control.

Table 3. Effect of *B. subtilis* and/or *C. vulgaris* levels on weight gain (WG), average daily gain (ADG), specific growth rate (SGR) and relative growth rate (RGR) of *O. niloticus* fingerlings.

Items	WG	ADG	SGR %	RGR
Control (T1)	27.65±0.52^{e}	0.28±0.01^{e}	2.25±0.04^{b}	8.24±0.52^{bc}
***B. subtilis* 2 g /Kg (T2)**	31.58±0.52^{c}	0.32±0.01^{c}	2.38±0.05^{ab}	9.33±0.53^{b}
***B. subtilis* 3 g/Kg (T3)**	33.76±0.38^{b}	0.34±0.003^{b}	2.43±0.05^{ab}	11.34±0.80^{a}
***C. vulgaris* 3gm/Kg (T4)**	29.16±0.70^{d}	0.30±0.01^{d}	2.41±0.06^{ab}	8.98±0.61^{b}
***C. vulgaris* 6gm/Kg (T5)**	33.95±0.47^{b}	0.35±0.004^{b}	2.43±0.05^{ab}	10.18±0.71^{ab}
***B. subtilis* 2 g /Kg + *C. vulgaris* 3gm/Kg (T6)**	36.75±0.48^{a}	0.37±0.004^{a}	2.55±0.03^{a}	11.72±0.93^{a}
***B. subtilis* 2 g /Kg + *C. vulgaris* 6gm/Kg (T7)**	37.18±0.47^{a}	0.38±0.004^{a}	2.54±0.04^{a}	11.45±0.74^{a}
***P*-Value**	<0.001	<0.001	<0.001	<0.001

± Means followed by the same letter in each column are not significantly different.

According to **Osei (2022)**, giving *Clarias gariepinus* diets treated with Bacillus resulted to a considerable rise in both final weight and weight gain. It has been demonstrated that *Bacillus sp.* gives the host organism essential nutrients including amino acids and vitamins K and B_{12}, which can enhance growth performance **(Liu *et al.*, 2012)**. At the conclusion of the two-month experiment, the probiotic groups showed the greatest increases in final weight and weight gain of *Clarias garriepinus*, with the rate of 10g/kg Bs group exhibiting the highest results. It follows that in order to have the optimum results, it is preferable to maintain a lower dose.

Table 3 displayed the SGR results as influenced by Bs and/or Cv. This table, which displays the average SGR values over the course of the experiment (14 weeks), reveals that, in comparison to fish fed the basal diet (T1 2.25%), the SGR of the Nile tilapia fish groups fed diets supplemented with Bs and/or Cv (T2, T3, T4, T5, T6, and T7) released significant ($P<0.01$) values of SGR were 2.38, 2.43, 2.41, 2.43, 2.55 and 2.54%, respectively.

Table 3 shows that the average RGR values for T1, T2, T3, T4, T5, T6, and T7 were 8.24, 9.33, 11.34, 8.98, 10.18, 11.72, and 11.45, respectively for Bs and/or Cv, as indicated. The fish groups fed the diets supplemented with Bs and/or Cv released the significant ($P<0.05$) values of RGR in comparison to fish fed the basal diet (T1).

In comparison to the control group, the growth rate and weight gain of *O. niloticus* rose as the amount of *C. vulgaris* in fish feeds increased, according to a study by **Saleh *et al.* (2022)**. Regarding **Khani *et al.* (2017)**, they demonstrated that a 5% *C. vulgaris* dietary supplement greatly boosted the growth of k fish.

Furthermore, the current findings concurred with the research conducted by **Adorian**

***et al.* (2018)**, which demonstrated that *Lates calcarifer* fed *Bacillus spp*. exhibited the best performance in condition factor. The results demonstrated that feeding catfish with Bacillus subtilis at levels that maximize feed utilization and fish development, with 10 g/kg Bs demonstrating the greatest benefit.

Table 4 displays the feed consumption results as impacted by adding Bs and/or Cv to tilapia diets. This table describes the feed intake during the entire experimental period (14 weeks). Table 4 results show that when Bs and/or Cv were added to the basal diet. fish in T5 was recorded the highest value (48.88 g) while fish in T1(control) was recoded the lowest value (43.48 g) of FI.

The differences in FI between the different treatments (T2, T3, T4, T5, T6, and T7) were not statistically significant, but the differences between each of these treatments and the control group (T1) were significant (P<0.05).

Table 4. Effect of *B. subtilis* and/or *C. vulgaris* levels on feed intake (FI), feed conversion ratio

Items	FI	FCR	PER
Control (T1)	43.48±0.76[b]	1.58±0.03[a]	2.12±0.04[d]
***B. subtilis* 2 g /Kg (T2)**	46.77±0.84[a]	1.36±0.02[c]	2.46±0.03[b]
***B. subtilis* 3 g/Kg (T3)**	46.31±0.98[a]	1.29±0.01[d]	2.60±0.03[a]
***C. vulgaris* 3gm/Kg (T4)**	47.41±1.12[a]	1.53±0.03[a]	2.19±0.04[d]
***C. vulgaris* 6gm/Kg (T5)**	48.88±1.06[a]	1.45±0.03[b]	2.32±0.04[c]
***B. subtilis* 2 g /Kg + *C. vulgaris* 3gm/Kg (T6)**	47.79±0.96[a]	1.31±0.02[cd]	2.54±0.04[ab]
***B. subtilis* 2 g /Kg + *C. vulgaris* 6gm/Kg (T7)**	48.44±1.05[a]	1.31±0.02[cd]	2.56±0.03[ab]
***P*-Value**	0.0029	<0.001	<0.001

By assessing the effect of probiotic and microalgae on the intestinal morphometry indices, the increased feed efficiency can be explained **(Bhowmik *et al*., 2009)**. The acquired results showed that the number of goblet cells and the length and width of the intestinal villi were both positively impacted by feeding fish both Bacillus and Spirulina. These findings support the hypothesis that probiotic bacillus and spirulina may have a role in improving intestinal barrier absorption ability, enabling the digestion of sufficient amounts of nutrients needed for fish body metabolic and biological processes **(Rombout *et al*., 2011)**. Additionally, the function of goblet cells in defending the intestinal walls against harmful microbes by secreting glycoproteins and antibacterial compounds is linked to their increasing quantity **(Knoop and Newberry, 2018)**.

According to **Dey (2023)**, there were notable alterations in growth rates and voluntary feed intake as a consequence of the algal inclusion diet. In contrast to the other groups, the control (0%) feed fed groups displayed a notable decline in growth performance. There were significant differences in the average daily gain (mg), growth rate, and SGR values across the feeding groups. Zebrafish (*Danio rerio*) fed diets including algae showed measurable weight gain, according to **Darwish *et al*. (2020)**. The outcome differs slightly from that of **Allam (2007)**, who demonstrated that a higher percentage of *Chlamydomonas sp*. inclusion (>10%) typically leads to reduced weight increase and SGR. However, other researchers found that, depending on the species and their feed consumption efficacy, partial substitution of dietary components up to 10 to 15% of the total feed content maximizes the growth rate in the case of algal feed formulation **(Valente *et al*., 2019)**.

The averages of the protein efficiency ratio (PER) and feed conversion ratio (FCR) were displayed in (Table, 4) results. FCR for fish fed on various experimental diets supplemented with Bs and/or Cv. As can be seen in this table, the control group's FCR values during the entire trial period (14 weeks) were the highest (worst) when compared to the other diets supplemented with various strains of Bs and/or Cv. Significant variations in FCR were observed by analysis of variance as a result of treatment effects when compared to control.

The results presented in Table 4 demonstrated that all feed additives significantly improved PER in comparison to the fish in the control group. The results of the analysis of variance (Table 5) demonstrated that the addition of Bs and/or Cv to the basal diets throughout the entire experimental period had a significant impact on the PER values. These values were also significantly better than those of the control diets, demonstrating the superiority of adding the tested Bs and/or Cv to the basal diet. The findings showed that there were notable differences between the control group and the various treatments. The PER data show that tilapia fish's protein consumption was greatly enhanced by Bs and/or Cv. The most costly feed ingredient, protein, is used for growth, and this helps to optimize its use. By improving the palatability of the diet, Chlorella/bacillus may also play a part in raising the feed conversion ratio and the protein efficiency ratio. Additionally, feeding Nile tilapia Bacillus sp. improved growth performance and feed utilization **(Elsabagh *et al*., 2018; Han *et al*., 2015)**, whereas, **Mahmoud *et al*. (2018)** observed that feeding spirulina also affected growth rates and feed efficiency. the fish fed spirulina supplementation had the highest growth compared to the control.

Results shown in Table 5 demonstrate that adding Bs and/or Cv to the basal diet had a substantial impact on hematocrit (Hct). Similarly, the levels of hemoglobin (Hb), mean corpuscular volume (MCV), and red blood cells (RBCs) showed significant fluctuation (P<0.05) but no discernible pattern. Fish fed the T6 and T7 diets (Bs 2 g /Kg + Cv 3gm/Kg and Bs 2 g /Kg + Cv 6gm/Kg) shown the greatest RBC, Hct, and Hb levels, while fish fed the diet T3 (BS 3gm/Kg) displayed the highest MCV levels.

Table 5. Effect of *B. subtilis* and/or *C. vulgaris* levels on some of blood hematological measurements of *O. niloticus* fingerlings.

Items	Hct	Hb	RBCs	MCV
Control (T1)	22.90±0.70[b]	9.85±0.25[c]	1.60±0.10[c]	143.5±3.5[ab]
***B. subtilis* 2 g /Kg (T2)**	22.15±0.15[b]	10.9±0.10[b]	1.75±0.05[bc]	130.0±6[bc]
***B. subtilis* 3 g/Kg (T3)**	26.30±1.30[a]	11.70±0.20[b]	1.75±0.05[bc]	149.50±9.50[a]
***C. vulgaris* 3gm/Kg (T4)**	23.20±0.60[b]	9.95±0.15[c]	1.75±0.05[bc]	144.5±4.50[ab]
***C. vulgaris* 6gm/Kg (T5)**	26.5±0.6[a]	11.35±0.15[b]	2.0±0[ab]	142.5±1.5[abc]
***B. subtilis* 2 g /Kg + *C. vulgaris* 3gm/Kg (T6)**	28.55±0.85[a]	13.1±0.6[a]	2.15±0.15[a]	131.5±4.50[abc]
***B. subtilis* 2 g /Kg + *C. vulgaris* 6gm/Kg (T7)**	27.65±0.45[a]	13.6±0.1[a]	2.05±0.05[a]	124.50±0.5[c]
***P*-Value**	0.0024	0.0002	0.0122	0.0012

± Means followed by the same letter in each column are not significantly different.

The blood components can be used to assess an animal's nutritional and pathological state based on the type, quantity, and toxicity of the food it consumes. The increased and decreased hematological and biochemical variables, when compared to the regular values, typically indicate the effect of feed additives on fish health **(Burgos Aceves *et al*., 2019; Dawood *et al*., 2019b)**. According to **Fazio (2019)**, the results showed that fish fed either bacillus algae or both bacillus and algae presented regular biochemical values that fell within the usual range for healthy fish.

According to **Dahiya *et al*. (2012)**, a rise in hematological parameters after Bacillus subtilis supplementation is a sign of healthy fish. According to **Farrell (2011)**, the main function of red blood cells is oxygen delivery. According to the study's findings, fish fed supplements with either bacillus or chlorella had considerably greater red blood cells (RBCs) than fish fed a control diet. These findings are consistent with those of earlier studies **(Azarin *et al*., 2014; Faramazi *et al*., 2011; Sharma and Sihag, 2013)**. This implies that fish fed diets treated with Bacillus have the ability to live well and thrive in challenging environments with low oxygen levels.

Fish in T1 (control) had the highest (P<0.05) ALT value (148.50 IU/L), according to data in Table (6), whereas fish from T7 (Bs 2 g/Kg + Cv 6 gm/Kg) had the lowest (P<0.05) ALT values, 76.0 IU/L. Fish from T1 (control) had the highest (P<0.05) AST value (70.0 IU/L), as this table demonstrates, whereas fish from T7 (Bs 2 g/Kg + Cv 6 gm/Kg) had the lowest (P<0.05) AST values, 42.0 IU/L. The effect of Bs and/or Cv on the serum creatinine and urea levels of Nile tilapia is shown in (Table, 6). As markers of renal function, urea and creatinine are thought to be crucial factors in determining the point at which the kidney is negatively impacted. Fish from T1 (control) had the highest urea value (8.85 mg/100 ml; P<0.05), while fish from T3 (Bs 3 g/Kg) had the lowest (6.3 mg/100 ml; P<0.05). The findings presented in Table (6) demonstrated a significant (P<0.05) drop in urea values as Bs and/or Cv levels increased. Data in Table (6) showed that fish of T2 (Bs 2 g /Kg) recorded the highest (P<0.05) creatinine value (1.60 mg/100 ml), while those of T5 (Cv 6gm/Kg) showed the lowest (P<0.05) creatinine values, was 0.95 mg /100ml.

AST and ALT are enzymes involved in several biochemical metabolic activities that interconvert amino acids to other metabolic intermediates, and an increase in their levels can indicate tissue damage, such as in chronic liver disease **(Abdollahi-Arpanahi *et al*., 2018; Babazadeh *et al*., 2011)**.

Table 6. Effect of *Bacillus subtilis* and/or *Chlorella vulgaris* levels on liver functions of *O. niloticus* fingerlings.

Items	ALT IU/L	AST IU/L	Urea (mg/100ml)	Creatinine (mg/100ml)
Control (T1)	148.50±3.50[a]	70.00±2[a]	8.85±0.65[a]	1.55±0.05[a]
***B. subtilis* 2 g /Kg (T2)**	120.50±1.50[c]	61.00±2[b]	7.75±0.15[ab]	1.60±0.10[a]
***B. subtilis* 3 g/Kg (T3)**	83.50±4.50[e]	50.00±1.02[cd]	6.30±0.20[c]	1.25±0.05[bc]
***C. vulgaris* 3gm/Kg (T4)**	130.00±2.50[b]	58.50±3.5[bc]	7.65±0.05[abc]	1.45±0.05[ab]
***C. vulgaris* 6gm/Kg (T5)**	93.50±1.5[d]	50.00±1[cd]	6.85±0.65[bc]	0.95±0.15[d]
***B. subtilis* 2 g /Kg + *C. vulgaris* 3gm/Kg (T6)**	80.00±1.01[e]	52.00±3.0[c]	6.53±0.20[bc]	1.20±.012[bcd]
***B. subtilis* 2 g /Kg + *C. vulgaris* 6gm/Kg (T7)**	76.00±1.06[e]	42.00±3.07[d]	6.55±0.35[bc]	1.03±0.11[cd]
***P*-Value**	0.0001	0.0012	0.0206	0.0048

± Means followed by the same letter in each column are not significantly different.

Nile tilapia fed Bs and/or Cv supplemented feed displayed a decrease in AST and ALT when compared to the control fish. This is thought to be related to their initial resistance to the bacillus and/or chlorella in the feed. Nevertheless, foods treated with Bacillus had lower levels of AST and ALT than control meals, suggesting that diets supplemented with Bs and/or Cv may assist enhance the health of the fish's liver, which may be related to their capacity to protect rather than damage the fish's liver. Measuring these enzymes is one of the most widely used methods to assess liver function **(Abdollahi-Arpanahi *et al*., 2018)**. There have been recent rumors that the cause of gall syndrome and liver enlargement in many farmed fish could be the buildup of contaminants **(Chai *et al*., 2016)**. As in the case of chronic liver disease, an increase in the levels of the enzymes aspartate transaminase (AST) and alanine transaminase (ALT), which interconvert amino acids with other metabolic intermediates and are involved in a variety of biochemical events in metabolism, can be an indication of tissue damage. **Liu *et al*. (2014)** state that because AST and ALT are cytoplasmic in origin and discharged into circulation (blood) upon cellular injury, they are sensitive biomarkers employed in the diagnosis of liver disease. .

Amylase and lipase, two digestive enzymes, were listed in (Table, 7) results. The table's results reveal that adding Bs and/or Cv to the basic diet had a substantial impact on amylase levels, and the variation in lipase was significant ($P<0.05$). The greatest levels of lipase and amylase were seen in fish fed the T7 diets (Bs 2 g/kg and Cv 6 gm/kg), at 50.05 and 41.30, respectively. Fish fed the food T2 (Bs 2 g/Kg) exhibited the lowest levels of lipase (19.35) and the lowest amylase level (16.45) was found in fish fed T1 diet (control). Probiotics are a class of bacteria that enhance the intestinal diversity, capacity for absorption and digestion, and local intestinal immunity within the fish's digestive system **(Pérez-Sánchez *et al*., 2014)**.

One of the most significant probiotic species employed in many fish species is *Bacillus* bacteria, whose spores are simple to add to aquafeed and have a reasonably long shelf life **(Ringo *et al*., 2018; Yılmaz *et al*., 2020)**. Another type of food additive is medicinal plants and their extracts, which can enhance the growth and health status of aquatic animals **(Dawood *et al*., 2018; Van Hai, 2015a)**.

In Nile tilapia (*O. niloticus*), the beneficial effects of *Bacillus spp*. are associated with the enhancement of the gastrointestinal tract digestion capacity, local intestinal immunity, and humoral and adaptive immune responses, as well as increased resistance against outbreaks and environmental stressors **(Chantharasophon *et al*., 2011; Han *et al*., 2015; Telli *et al*., 2014)**.

Table 7. Effect of *B. subtilis* and/or *C. vulgaris* levels on digestive enzymes and immunological variables of *O. niloticus* fingerlings

Table 8 displays the outcomes of the immunological variables immunoglobulin A (IgA),

Items s	Amylase (U/L)	Lipase (U/L)
Control (T1)	16.45±1.65^{d}	20.20±1.9^{e}
***B. subtilis* 2 g /Kg (T2)**	25.4±3.5^{c}	19.35±1.55^{e}
***B. subtilis* 3 g/Kg (T3)**	31.35±1.45^{b}	31.40±2.5^{d}
***C. vulgaris* 3gm/Kg (T4)**	24.05±1.15^{c}	24.45±1.75^{e}
***C. vulgaris* 6gm/Kg (T5)**	32.65±0.75^{b}	37.50±1.6^{c}
***B. subtilis* 2 g /Kg + *C. vulgaris* 3gm/Kg (T6)**	36.20±0.3^{ab}	43.85±1.05^{b}
***B. subtilis* 2 g /Kg + *C. vulgaris* 6gm/Kg (T7)**	41.30±1.2^{a}	50.05±1.25^{a}

± Means followed by the same letter in each column are not significantly different.

The quantity of total proteins, enzymes, antibodies, and immune reactions in the blood is shown by the detection of immunoglobulin (IgA, IgM and IgG) (**Magnadóttir, 2006**). Interestingly, the current study found that tilapia fed Bs and/or Cv had increased levels of IgA, IgM, and IgG. This finding can be explained by the effect of Bacillus and Chlorella on boosting the immune response of Nile tilapia. Nile tilapia have been shown to exhibit elevated IgG levels in response to Bacillus or *S. platensis* (Han ***et al*., 2015; Mahmoud *et al*., 2018**). The tilapia body's improved immunity, most likely as a result of boosting the local intestine immunity, is directly responsible for the higher IgA, IgM, and IgG levels **(Kiron, 2012)**.

According to a study by **Guo *et al*. (2009)**, the frequency of probiotic administration significantly affects their efficacy. During the culture period, a daily probiotic addition is better than an application every other day. Like other immunostimulant medicines, short-term cyclic probiotic feeding approaches may benefit the hosts **(Bricknell and Dalmo, 2005)**. Examples of such treatments include cyclically switching between probiotic-supplemented and un supplemented meals for shorter intervals of time. Short-term use of this application may result in direct benefits during the supplemental feeding phase. Probiotics provide defense against acute infections throughout the stage without supplementation, and they might also generate some level of immunostimulation if stomach probiotic populations

immunoglobulin M (IgM), and immunoglobulin G (IgG) in relation to Bs and/or Cv. The table's findings demonstrated that, in comparison to fish given the basal diet (T1), the Nile tilapia fish groups (T2, T3, T4, T5, T6, and T7) fed diets supplemented with Bs and/or Cv emitted the significant ($P<0.01$) values of Immunological Variables.

The fish fed on T6 diets (Bs 2 g/kg + Cv 3 g/kg) recorded the highest levels of IgA, IgM, and IgG variables, measuring 93.20, 65.03, and 48.11, respectively, according to table 8. While these variables were lowest in fish fed diet T1 (control) (36.40, 47.60, and 30.80, respectively) for IgA, IgM and IgG.

were stable for a few weeks **(Balcázar and Rojas-Luna, 2007).**

Table 8. Effect of *Bacillus subtilis* and/or *Chlorella vulgaris* levels on immunological variables of *O. niloticus* fingerlings.

Items	IgA	IgM	IgG
Control (T1)	36.40±0.5^{c}	47.60±2.30^{e}	30.80±1.1^{d}
Bacillus subtilis 2 g /Kg (T2)	40.45±1.55^{c}	57.7±1^{c}	35.1±1.5^{c}
Bacillus subtilis 3 g/Kg (T3)	69.10±2.2^{b}	60.30±0.8^{c}	42.60±1.4^{b}
Chlorella vulgaris 3gm/Kg (T4)	43.70±0.7^{c}	52.15±0.45^{d}	36.55±1.35^{c}
Chlorella vulgaris 6gm/Kg (T5)	73.05±4.05^{b}	61.15±0.25^{cb}	44.85±0.05^{ab}
Bacillus subtilis 2 g /Kg + Chlorella vulgaris 3gm/Kg (T6)	93.20±5.1^{a}	65.03±0.1^{a}	48.11±0.9^{a}
Bacillus subtilis 2 g /Kg + Chlorella vulgaris 6gm/Kg (T7)	91.45±5.15^{a}	64.55±0.85^{ab}	47.95±1.65^{a}
P-Value	<0.001	<0.001	<0.001

± Means followed by the same letter in each column are not significantly different.

Conclusion:

Alternatives for enhancing fish development and health have surfaced, including *B. subtilis* and *C. vulgaris*. Commercially available Bs and CV are growth enhancers that can improve fish hematological parameters, according to the current study's findings. When compared to a control diet, *O.*

niloticus monosex fingerlings' growth was improved when their food was supplemented with Bs, Cv, or both. Furthermore, dietary supplements for *O. niloticus* monosex include Bs and/or Cv, with the highest effect on hematological indicators, considerably improved plasma chemistry, hematological parameters and organism's liver health as compared to the control diet.

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REFERENCES

Abdel-Latif, H. M. R., Abdel-Tawwab, M., Dawood, M. A. O., Menanteau-Ledouble, S. and El-Matbouli, M. (2020). Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: a review. Rev. Fish. Sci. Aquac. 1–28. https://doi.org/ 10.1080/23308249.2020.1758899.

Abdel-Latif, H. M. R., Dawood, M. A. O., Menanteau-Ledouble, S. and El-Matbouli, M. (2020). The nature and consequences of co-infections in tilapia: a review. J. Fish Dis n/a.

Abdollahi-Arpanahi, D., Soltani, E., Jafaryan, H., Soltani, M., Naderi-Samani, M. and Campa-Córdova, A. I. (2018). Efficacy of two commercial and indigenous probiotics, *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, immuno-physiology and resistance response of juvenile white shrimp (*Litopenaeus vannamei*). Aquaculture, 496(1), 43-49.

Adorian, T. J., Jamali, H., Farsani, H. G., Darvishi, P., Hasanpour, S., Bagheri, T. and Roozbehfar, R. (2018). Effects of Probiotic Bacteria *Bacillus* on Growth Performance, Digestive Enzyme Activity, and Hematological Parameters of Asian Sea Bass, Lates calcarifer (Bloch). Probiotics and Antimicrobial Proteins, 11(1), 248–255. https://doi.org/10.1007/s12602-018-9393-z

Akanmu, O. A. (2018). Probiotics, an alternative measure to chemotherapy in fish production. Probiotics: Current Knowledge and Future Prospects. pp. 151.

Allam, H. Y. (2007). Physiological effects of some additives on growth, blood constituents and immunity in Nile tilapia (*Oreochromis niloticus*) (Doctoral dissertation, Thesis, Faculty of Agriculture, University of Assiut).

AOAC, A. (2000). Association of Official Analytical Chemists. Official Methods of Analysis, AOAC, Arlington, VA, USA.

Azarin, H., Aramli, M. S., Imanpour, M. R. and Rajabpour, M. (2014). Effect of a Probiotic www.udsspace.uds.edu.gh Containing *Bacillus licheniformis* and *Bacillus subtilis* and Ferroin Solution on Growth Performance, Body Composition and Haematological Parameters in Kutum (*Rutilus frisii kutum*) Fry. Probiotics and Antimicrobial Proteins, 7(1), 31–37. https://doi.org/10.1007/s12602-014-9180-4.

Babazadeh, D., Vahdatpour, T., Nikpiran, H., Jafargholipour, M. A. and Vahdatpour, S. (2011). Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (*Coturnix japonica*). Indian Journal of Animal Sciences, 81(8), 870.

Balasubramaniam, P., and Malathi, A. (1992). Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. Journal of postgraduate medicine. 38: 8.

Balcázar, J. L. and Rojas-Luna, T. (2007). Inhibitory activity of probiotic *Bacillus subtilis* UTM 126 against vibrio species confers protection against vibriosis in juvenile shrimp (*Litopenaeus vannamei*). Curr Microbiol 55:409–12

Becker, E. W. (2007). "Micro-algae as a source of protein". Biotechnology Advances 25 (2): 207–10. doi:10.1016/j.biotechadv.2006.11.002. PMID 17196357.

Bhowmik, D., Dubey, J, and Mehra, S. (2009). Probiotic efficiency of *Spirulina platensis*-stimulating growth of lactic acid bacteria. World J. Dairy Food Sci. 4, 160–163. Bonga, W.E.S., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Blaxhall, P. and Daisley, K. (1973). Routine haematological methods for use with fish blood. Journal of fish biology. 5: 771-781.

Bonga, W. E. S. (1997). The stress response in fish. Physiol. Rev. 77, 591–625.

Bricknell, I. and Dalmo, R. A. (2005). The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol 19:457–472.

Burgos-Aceves, M. A., Lionetti, L. and Faggio, C. (2019). Multidisciplinary hematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Sci. Total Environ.

Cao, Y., Liu, H., Qin, N., Ren, X., Zhu, B. and Xia, X. (2020). Impact of food additives on the composition and function of gut microbiota: a review. Trends Food Sci. Technol. 99, 295–310.

Caraway, W. T. (1959). A stable starch substrate for the determination of amylase in serum and other body fluids. American Journal of Clinical Pathology. 32: 97-99.

Chai, P. C., Song, X. L., Chen, G. F., Xu, H., and Huang, J. (2016). Dietary supplementation of probiotic Bacillus PC465 isolated from the gut of *Fenneropenaeus chinensis* improves the health status and resistance of *Litopenaeus vannamei* against white spot syndrome virus. Fish & shellfish immunology, 54, 602-611.

Chantharasophon, K., Warong, T., Mapatsa, P., and Leelavatcharamas, V. (2011). High potential probiotic Bacillus species from gastro-intestinal tract of Nile tilapia *(Oreochromis niloticus).* Biotechnology 10, 498–505.

Cicia, A. M., Schlenker, L. S., Sulikowski, J. A., and Mandelman, J. W. (2012). Seasonal variations in the physiological stress response to discrete bouts of aerial exposure in the little skate, *Leucoraja erinacea.* Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 162(2), 130–138.

Dacie, J. and Lewis, S. (1991). Miscellaneous tests. Practical haematology. 7th Ed. London, Churchill Livingstone: 227-257.

Dahiya, T., Sihag, R. C. and Gahlawat, S. K. (2012). Effect of Probiotics on the Haematological Parameters of Indian Magur (*Clarias batrachus* L.). Journal of Fisheries and Aquatic Science, 7(4), 279–290. https://doi.org/10.3923/jfas.2012.279.290

Darwish, R., Gedi, M. A., Akepach, P., Assaye, H., Zaky, A. S. and Gray, D. A. (2020). *Chlamydomonas reinhardtii* is a potential food supplement with the capacity to outperform *Chlorella* and *Spirulina*. Applied Sciences. 10(19): 6736.

Dawood, M. A. O., Abo-Al-Ela, H. G., and Hasan, M. T. (2020). Modulation of transcriptomicprofile in aquatic animals: probiotics, prebiotics and synbiotics scenarios. Fish Shellfish Immunol. 97, 268–282.

Dawood, M. A. O. and Koshio, S. (2016). Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture 454, 243–251.

Dawood, M. A. O. and Koshio, S. (2020). Application of fermentation strategy in aquafeed for sustainable aquaculture. Rev. Aquac. 12, 987–1002.

Dawood, M. A. O., Koshio, S., Abdel-Daim, M. M. and Van Doan, H. (2019a). Probiotic application for sustainable aquaculture. Rev. Aquac. 11, 907–924.

Dawood, M. A. O., Koshio, S. and Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac. 10, 950–974.

Dawood, M. A. O., Koshio, S., Ishikawa, M., and Yokoyama, S. (2015). Interaction effects of dietary supplementation of heat-killed Lactobacillus plantarum and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, Pagrus major. Fish Shellfish Immunol. 45, 33–42.

Dawood, M. A. O., Magouz, F. I., Salem, M. F. I., and Abdel-Daim, H. A. (2019b). Modulation of digestive enzyme activity, blood health, oxidative responses and growth-related gene expression in GIFT by heat-killed Lactobacillus plantarum (L-137). Aquaculture 505, 127–136.

De Punder, K., and Pruimboom, L. (2015). Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. Front. Immunol. 6, 223.

Del Campo, J. A., Garcia-Gonzalez, M. and Guerrero, M. G. (2007). Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Applied Microbiology and Biotechnology 74, 1163-1174.

Dey, S. K. (2023). Evaluation of Growth, Survival and Hemato-Biochemical Indices of Common Carp (*Cyprinus Carpio*) Through Partial Replacement of Fish Meal by Microalgae Powder Isolated from South-Eastern Coast of Bangladesh. M. Sc. Thesis, Faculty of Fisheries Chattogram Veterinary and Animal Sciences University Khulshi, Chattogram-4225, Bangladesh.

El Asely, A. M., Reda, R. M., Salah, A. S., Mahmoud, M. A., and Dawood, M. A. O. (2020b). Overall performances of Nile tilapia (*Oreochromis niloticus*) associated with using vegetable oil sources under suboptimal temperature. Aquac. Nutr

El Asely, A., Amin, A., Abd El-Naby, A. S., Samir, F., El-Ashram, A. and Dawood, M. A. O. (2020a). Ziziphus mauritiana supplementation of Nile tilapia (*Oreochromis niloticus*) diet for improvement of immune response to *Aeromonas hydrophila* infection. Fish Physiol. Biochem. https://doi.org/10.1007/s10695-020-00812-w.

Elsabagh, M., Mohamed, R., Moustafa, E. M., Hamza, A., Farrag, F., Decamp, O., Dawood, M. A. O. and Eltholth, M. (2018). Assessing the impact of Bacillus strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, (*Oreochromis niloticus*). Aquac. Nutr. 24, 1613–1622.

FAO. (2018). Aquaculture Department, the State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome, pp. 2430.

Faramazi, M., Kiaalvandi, S., Lashkarbolooki, M. and Iranshahi, F. (2011). The Investigation of Lactobacillus acidophilus as probiotics on growth performance and disease resistance of www.udsspace.uds.edu.gh 58 rainbow trout (*Oncorhynkus mykiss*). American-Eurasian J Sci Res, 6, 32–38.

Farrell, A. P. (2011). Blood Cellular Composition of the Blood. In Encyclopedia of Fish Physiology (Vol. 2). Elsevier Inc. https://doi.org/10.1016/B978-0-12-374553-8.00125-8

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500, 237–242.

Goering, H. K. and Van Soest, P. J. (1970). Forage Fiber Analysis (Apparatus Reagents, Procedures and Some Applications). Agriculture Handbook. United States Department of Agriculture, Washington DC

Guccione, A., Biondi, N., Sampietro, G., Rodolfi, L., Bassi, N. and Tredici, M. R. (2014). *Chlorella* for protein and biofuels: from strain selection to outdoor cultivation in a green wall panel photobioreactor. Biotechnology for Biofuels 7, 84.

Guo, J. J., Liu, K. F., Cheng, S. H., Chang, C. I., Lay, J. J., Hsu, Y. O. and Chen, T. I. (2009). Selection of probiotic bacteria for use in shrimp larviculture. Aquaculture Research, 40(5), 609-618.

Han, B., Long, W. q., He, J. y., Liu, Y. j., Si, Y. q. and Tian, L. x. (2015). Effects of dietary *Bacillus licheniformis* on growth performance, immunological parameters, intestinal morphology and resistance of juvenile Nile tilapia (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunol. 46, 225–231.

Herrera, M., Mancera, J. M. and Costas, B. (2019). The use of dietary additives in fish stress mitigation: comparative endocrine and physiological responses. Front Endocrinol (Lausanne) 10, 447.

Juneja, A., Ceballos, R. M. and Murthy, G. S. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A Review. Energies 6, 4607-4638.

Khani, M., Soltani, M., Shamsaie, M. M., Foroudi, F. and Ghaeni, M. (2017). The effects of *Chlorella* vulgaris supplementation on growth performance, blood characteristics and digestive enzymes in Koi (*Cyprinus carpio*). Iranian Journal of Fisheries Sciences, 16(2), 832- 843.

Kiron, V. (2012). Fish immune system and its nutritional modulation for preventive health care. Anim. Feed Sci. Technol. 173, 111–133.

Knoop, K. A., and Newberry, R. D. (2018). Goblet cells: multifaceted players in immunity at mucosal surfaces. Mucosal Immunol. 11, 1551–1557.

Kothari, D., Patel, S. and Kim, S. K. (2019). Probiotic supplements might not be universally effective and safe: a review. Biomed. Pharmacother. 111, 537.

Liu, C. H., Chiu, C. H., Wang, S. W. and Cheng, W. (2012). Dietary administration of the probiotic, *Bacillus subtilis* E20, enhances the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. Fish & Shellfish Immunology ,V. 33 (4), P. 699-706.

Liu, H., Li, Z., Tan, B., Lao, Y., Duan, Z., Sun, W. and Dong, X. (2014). Isolation of a putative probiotic strain S12 and its effect on growth performance, non-specific immunity and disease-resistance of white shrimp, *Litopenaeus vannamei*. Fish & shellfish immunology, 41(2), 300-307.

Magnadóttir, B. J. F. (2006). Innate Immunity of fish (overview). Fish Shellfish Immunol. 20, 137–151.

Mahmoud, M. M. A., El-Lamie, M. M. M., Kilany, O. E. and Dessouki, A. A. (2018). Spirulina (*Arthrospira platensis*) supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (*Oreochromis niloticus*) challenged with Pseudomonas fluorescens. Fish Shellfish Immunol. 72, 291–300.

Moss, D. W. and Henderson, A. R. (1999). Digestive enzymes of pancreatic origin. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; p. 689-708).

National Research Council (NRC). (1993). Nutrient requirements of fish. National Academy Press, Washington DC.

Osei, S. A. (2022). Performance of Bacillus Subtilis in Improving Growth, Haematology and Liver Health of The African Catfish (*Clarias Gariepinus*, Burchell 1822). M. Sc. Thesis. Faculty Of Biosciences, University for Development Studies, Tamale.

Pérez-Sánchez, T., Ruiz-Zarzuela, I., de Blas, I. and Balcázar, J. L. (2014). Probiotics in aquaculture: a current assessment. Rev. Aquac. 6, 133–146.

Ringo, E., Hossein, S., Ghosh, K., Doan, H. V., Beck, B. R. and Song, S., (2018). Lactic acid bacteria in finfish–an update. Front. Microbiol. 9, 1818.

Rombout, J. H. W. M., Abelli, L., Picchietti, S., Scapigliati, G. and Kiron, V. (2011). Teleost intestinal immunology. Fish Shellfish Immunol. 31, 616–626.

Saleh, H. A., Gaber, H. S., El-Khayat, H. M. M., Abdel-Motleb, A., Mohammed, W. A., and Okasha, H. (2022). Influences of Dietary Supplementation of *Chlorella vulgaris* and *Spirulina platensis* on Growth-Related Genes Expression and Antioxidant Enzymes in *Oreochromis niloticus* Fish Exposed to Heavy Metals. Aquaculture Studies, 22(2), AQUAST793. http://doi.org/10.4194/AQUAST793

Santos, L. and Ramos, F. (2018). Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. Int. J. Antimicrob. Agents 52, 135–143.

SAS. (2012). Institute Inc. SAS/STAT Statistics user's guide. Statistical analytical system, 5th rev ed. Cary, NC, USA.

Sharma, P. and Sihag, R. C. (2013). Pathogenicity test of bacterial and fungal fish pathogens in *Cirrihinus mrigala* infected with EUS disease. In Pakistan Journal of Biological Sciences (Vol. 16, Issue 20, pp. 1204–1207). https://doi.org/10.3923/pjbs.2013.1204.1207

Shelby, R. A., Lim, C., Yildirim-Aksoy, M. and Delaney, M. A. (2006). Effects of Probiotic Diet Supplements on Disease Resistance and Immune Response of Young Nile Tilapia, *Oreochromis niloticus*. Journal of Applied Aquaculture, 18(2), 23–34. https://doi.org/10.1300/J028v18n02_02

Stoskopf, M. (1993). Fish medicine. Philadelphia. London. Toronto. Montreal. Sydney. Tokyo. WB Saunders company. Harcourt Brace Jovanovich Inc.

Telli, G. S., Ranzani-Paiva, M. J. T., Dias, Dd. C., Sussel, F. R., Ishikawa, C. M. and Tachibana, L. (2014). Dietary administration of *Bacillus subtilis* on hematology and non-specific immunity of Nile tilapia *Oreochromis niloticus* raised at different stocking densities. Fish Shellfish Immunol. 39, 305–311.

Valente, A., Iribarren, D. and Dufour, J. (2019). How do methodological choices affect the carbon footprint of microalgal biodiesel-A harmonised life cycle assessment. Journal of Cleaner Production. 207:560-568.

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: a review. Aquaculture 446, 88–96.

Wilkinson, J., Baron, D., Moss, D. and Walker, P. (1972). Standardization of clinical enzyme assays: a reference method for aspartate and alanine transaminases. Journal of Clinical Pathology. 25: 940.

Yılmaz, S., Ergun, S., Yigit, M. and Çelik, E. Ş. (2020). Effect of combination of dietary *Bacillus subtilis* and trans-cinnamic acid oninnate immune responses and resistance of rainbow trout, *Oncorhynchus mykiss* to Yersinia ruckeri. Aquac. Res. 51, 441–454.

Yilmaz, S., Yigit, N. Ç. S. E. M. and Çelik, E. Ş. (2019). Combined effects of dietary *Bacillus subtilis* and Trans-cinnamic acid on growth performance, whole body compositions, digestive enzymes and intestinal bacteria in Rainbow trout (*Oncorhynchus mykiss*). J. Zoolog. Syst. Evol. Res. 1.

الملخص العربي

تأثير إضافة البكتيريا العصوية وطحلب الكلوريلا في العلائق على أداء النمو والمتغيرات الدموية والبيوكيميائية لإصبعيات أسماك البلطي النيلي وحيد الجنس

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تهدف هذه الدراسة إلى تقييم التأثيرات التآزرية المحتملة للبكتيريا العصوية وطحلب الكلوريلا في العلائق على أداء النمو، الاستفادة من الأعلاف، المتغيرات الدموية والبيوكيميائية والاستجابة المناعية لإصبعيات البلطي النيلي وحيد الجنس. تم تقسيم 210 إصبعية من البلطي النيلي (بمتوسط وزن 3.55 جرام وضعت في 21 حوضًا زجاجيًا / ثلاث مكررات لكل معاملة) إلى ستة أنظمة غذائية تجريبية غنية بـالبكتيريا العصوية (3 أو 6 جم لكل كجم من النظام الغذائي) أو طحلب الكلوريلا (2 و 3 جم لكل كجم من النظام الغذائي) أو كل من منهما لمدة 14 أسبوعًا، بالإضافة إلى عليقة التحكم. أظهرت البيانات أن الأسماك التي تغذت على البكتيريا العصوية و/أو طحلب الكلوريلا كانت ألأفضل من حيث وزن الجسم، زيادة الوزن ومعدل النمو النوعى ومعدل التحويل الغذائى عن المجموعة الضابطة. وأظهرت المتغيرات الدموية والبيوكيميائية اختلافات بين المجموعات ذات القيم الطبيعية.
يوضح ما سبق أهمية التغذية بوجبات غنية بالبكتيريا العصوية وطحلب الكلوريلا لتحسين أداء النمو والصحة العامة لأسماك البلطي النيلي وحيد الجنس