Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Nutritional Impact of Incorporating Probiotic Bacteria in Fingerlings Nile Tilapia (*Oreochromis niloticus*) Fish Diets

Doaa K. Khames[1], Ali S.M. El-Nadi[2], Hamed A.A. Omer[2] and Samah A.A. Abd El-Hameed[1]

[1] *Fish Nutrition and Feed Technology Department, Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abu-Hammad 44662, Agriculture Research Center, Giza, Egypt.*

[2] *Animal Production Department, Biological Agriculture Research Institute, National Research Centre, Dokki, Giza 12622, Egypt*.

Abstract

A TOTAL number of 150 fingerlings Nile tilapia initial weights of 20.48±0.036g used to investigate the impact of incorporating probiotic bacteria in fish diets on their performance, feed utilization, blood parameters, fish body composition and energy retention (ER) & protein productive value (PPV) % of Nile tilapia fish. Fish were distributed in 15 aquariums and 10 fish put in each aquaria 100 litter Two probiotic strains (*B. toyonensis and G. stearothermophilus*) were added at 1×10^5 CFU(Colony Forming Unit) ml^{-1} and 2×10^5CFU ml^{-1} on control diet. The results showed that dietary treatments had significantly affecting on water quality that includes water temperature, dissolved oxygen, ammonia (NH_4), electric conductivity, total dissolved solids and nitrate. Values of FW, TBWG, ADG, SGR and RGR significantly Colony Forming Unit increased and SR values were improved from 80% to 100% and mortality rate % improved from 20% in control to Zero% with inclusion probiotic bacteria in the diets. Dietary treatments significantly improved values of feed conversion ratio (FCR). Values of WBC's cell count, AST and ALT significantly decreased, but uric acid decreased. Values of total protein, Globulin, hemoglobin concentration, RBC's cell countin creased. Values of nitric oxide (NO), nitro blue tetrazoline (NBT), lysozyme (LYZO), catalse enzyme (CAT) and super oxide dimutase (SOD) significantly gradually increased with increasing level of probiotic bacteria introduced compared to the control. Inclusion probiotic bacteria significant increased values of Organic matter, Crude protein, Ether extract and gross energy, but ash content significantly decreased. Both values of ER% and PPV% significantly increased. From the present results it can be mentioned that probiotic bacteria can be used in fish diets because it had no any adverse effects and improvement values of growth performance, feed utilization, blood content, fish body composition and ER and PPV%.

Keywords: Probiotic bacteria, Feed utilization, Blood constituents, Antioxidants, Immune parameters energy retention,

Introduction

The harmful ecological impacts of chemical and synthetic medications, including antibiotics, have been well documented. These substances can lead to the development of mutagenic, antibiotic-resistant bacterial strains and negatively affect fish health and aquatic ecosystems [1,2]. In recent years, increasing attention has been directed toward the use of eco-friendly alternatives, such as probiotic supplements, which have shown promising results in enhancing growth performance, antioxidant status, and immune responses in fish [3].

Probiotics are now considered one of the most effective alternative strategies for immunological prophylactic management in aquaculture, as highlighted by [4]. Several *Bacillus* species such as *B. subtilis*, *B. cereus*, *B. coagulans*, *B. clausii*, *B. megaterium*, *B. licheniformis*, *B. circulans*, *B. aerius*, and *B. polymyxa* are commonly used probiotics for promoting fish growth and health [5]. Furthermore,

*Corresponding authors: Ali S.M. El-Nadi, E-mail: ali_elnadi_nrc@yahoo.comTel.: 9647504592159
(Received 26 May 2025, accepted 23 July 2025)
DOI: 10.21608/ejvs.2025.389364.2871

studies by [6,7] demonstrated the effectiveness of *Bacillus* spp. in protecting fish against infectious diseases and enhancing their growth performance.

Probiotics are also known to produce inhibitory substances that help prevent pathogen invasion and support microbial balance within the aquatic environment [8–10]. Additionally, [11] reported that incorporating probiotics into tilapia rearing water improves both growth and immune response. Recent findings by [12,13] have also revealed the positive role of *Geobacillusstearothermophilus* in enhancing fish productivity and improving the physicochemical properties of water quality.

Based on this background, the present study was conducted to investigate the effects of incorporating two probiotic strains *Bacillus toyonensis* and *Geobacillusstearothermophilus* at three levels (0, 1×10^5 CFU/ml, and 2×10^5 CFU/ml) on water quality, productive performance, feed utilization, hematological and biochemical parameters, antioxidant and immune responses, body composition, energy retention, and protein productive value in Nile tilapia (*Oreochromisniloticus*).

Material and Methods

This work was carried out at Fish Laboratory belonging to Fish Nutrition and Feed Technology Department, Central Lab. for Aquaculture Research, Agriculture Research Center, Abbassa, Abu-Hammad 44662, Sharkia, Egypt in co-operation work with Animal production Department, Biological Agriculture Research Institute, National Research Centre and Fish Health and Management Department, Central Lab. for Aquaculture Research, Agriculture Research Center, Abbassa, Abu-Hammad 44661, Sharkia, Egypt.

This work was done to established the impact incorporated two isolated probiotic strains (*B. toyonensis*and*G. stearothermophilus*) at two levels (Zero, 1×10^5 CFU ml^{-1} and 2×10^5 CFU ml^{-1})on water quality, productive performance, feed utilization, fish body composition, blood constituents, antioxidants & immune parameters and energy retention (ER) & protein productive value (PPV) % of Nile tilapia (*Oreochromisniloticus*) fish. The probiotics that used added on the control diet. Also need to refine material and method

Experimental unit

A total of 150 Nile tilapia (*Oreochromisniloticus*) fingerlings, with an initial average body weight of 20.48 ± 0.036 g, were used after an acclimation period. The fish were randomly allocated into 15 glass aquaria (100 L each), with 10 fish per aquarium. Each treatment group consisted of three replicates, with each replicate represented by a single aquarium.

Experimental diets

The design of the different experimental group diets and chemical analysis of the commercial diet presented in Table (1).

All tilapia fish were fed a commercial diet (30% protein) two times daily at (8:00 a.m. and 16:00 p.m) at level 3% of live body weight and experimental feeding period continuous for 70 days extended approximately from first February 2023 to middle April, 2023.

The lower concentration was used based on prior described by [14,15], meanwhile, the higher level was used as noted by [11].

Water quality measurements

Recorded water quality measurements includes water temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), conductivity (EC), total ammonia (TAN), Nitrites (NO2- µ M^{-1}) and Nitrate (NO3- µ M^{-1}). The water temperature and dissolved oxygen were measured daily by oxygen meter (Lutron model Do- 5509, Taiwan) and the pH values were recorded by digital pH meter (Hanna model PHEP, USA). Total ammonia concentration was measured by comparison apparatus using HACH kits (Hach Co., Loveland, Colorado, USA). The percentages of unionized ammonia (NH_4) were calculated from multiplying the total ammonia value by the appropriate factor according to the following equation:

Ammonia concentration (mg/L as NH3) = A/ 100 x 1.2 x total ammonia.

Where A is a coefficient related to water pH and temperature.

Parameters of growth performance

Body weight gain (BWG) = Final weight - Initial weight.
Survival rate (SR %) = Number of fish at final / Number of fish at start x100.
Specific growth rate (SGR) =
[In final weight (g) - In initial weight (g)] / Experimental days *100

Calculation of feed conversion ratio (FCR)

FCR = total dry matter intake, (TDMI), g / total body weight gain (TBWG), g.

Calculation of crude protein efficiency ratio (CPER)

(PER) = total body weight gain (TBWG), g / total crude protein intake (TCPI), g.
Feed efficiency (FE %) = [weight gain (g) / feed intake (g)]
Protein productive value (PPV %) = [PR_1– PR_0 / PI] 100.
Where: PR_1 = is the total fish body protein at the end of the experiment.

PR_0 = is the total fish body protein at the start of the experiment.
PI = Protein intake.
Energy retention percentages (ER %)
The energy retention percentage was calculated according the following equation:
Energy retention (ER %) = $E-E_0 / E_F X 100$
Where: E= the energy in fish carcass (kcal) at the end of the experiment.
E_0= the energy in fish carcass (kcal) at the start of the experiment.
E_F = the energy (kcal) in feed intake.

Blood sampling

Blood samples were collected from the caudal vein of the 24 fish by a 3ml syringe after anesthetizing of fish using clove oil (0.5 ml L^{-1}). Then, blood samples were kept in clean and dry centrifuge tubes, at room temperature, till clotting, then centrifuged at 3000 rpm for 15 min. Serum was separated, collected, and then stored at -20°C until biochemical analysis.

Body composition

In the beginning 10 fish used meanwhile, at the end 5 fish from each treatment were randomly chosen to determine their whole body composition.

Analytical procedures

Analysis of basal diet and fish body composition analyzed according to [16]

Serum total proteins determined described by according [17-19], albumin evaluated as according by[20-24], globulin calculated by subtracting albumin concentration from total protein concentration, the albumin/globulin (A/G) ratio was calculated by dividing albumin values by globulin values.Bloodhemoglobin was estimated according to the method that described by [25-27].Red blood cell counts (RBC's) and white blood cell (RBC's) count. of collected blood samples were estimated according to method described by [28]. Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determined as described by [29-31].Both uric acid and creatinine determined according to [22]. All evaluations were estimated using commercial biochemical kits (Spectrum-diagnostics, Egypt). Each biochemical parameter was calorimetrically analyzed according to its manufacturer's instructions using an Agilent Cary UV-Vis spectrophotometer (100/300 Series).

Antioxidant and immune parameters

Superoxide dismutase (SOD) and catalase (CAT) concentration evaluated using commercial colorimetric kits as described by [32-34]. Nitric oxide (NO) level determined according the method shown by [35]. The respiratory burst test was determined as ascribed by[36]method, whereas 0.1 ml of the heparinized blood was mixed with an equal volume of 0.2% nitro blue tetrazolium (NBT) solution in a micro titer plate. Following 30-min incubation at room temperature (25 °C), 0.05 ml of the mixture was added to 1 ml N, N dimethylformamide. The solution was mixed and centrifuged at 3000 rpm for 5 min. The optical density was measured at 540 nm in a spectrophotometer. The serum lysozyme activity was estimated via applying turbidity procedure [37]. Establishing the calibration curve by prepared a serial dilution of the standard lysozyme from hen egg white (Fluka, Switzerland) and mixed with *Micrococcus lysodeikticus*(ATCC NO. 1698 Sigma) suspension. Ten micro liters of serum or standard solution was blended with 200 µl of *Micrococcus* suspension (35 mg of *Micrococcus* dry powder per 95 ml of 1/15 M phosphate buffer + 0.5 ml of NaCl solution). The alterations in the extinction were determined at 546 nm via quantifying the extinction directly after supplying the solution which stimulate the lysozyme (start of the reaction) and after 20-min incubation of the preparation under examination at 40 °C (end of the reaction). The lysozyme values were calculated based on the extinction induced and calibration curve.

Analytical data

The gross energy (kcal/ kg DM) of experimental diets and body composition of tested group fish were calculated [38,39] using the following values, each g CP= 5.65 Kcal, g EE= 9.40 Kcal and g CF & NFE = 4.15 Kcal.

Metabolizable energy (ME) calculated according to [40]using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate. Alsoprotein energy ratio (mg CP/ Kcal ME): Calculated according to [40].

Statistical analysis

The collected data were subjected to statistical analysis as one-way analysis of variance using the general linear model procedure of [41].[42]was used to separate means when the dietary treatment effect was significant according to the following model:

$Y_{ij}=\mu+ T_i + e_{ij}$

Where: Y_{ij}= Observation,

μ = the overall mean,

T_i =the effect of incorporated probiotic bacteria for i = 1 to 5, G_1: fish received control diet, G_2: fish received diet contained 1×10^5 CFU ml^{-1} of *B. toyonensis* (BT1), G_3: fish received diet contained 2×10^5 CFU ml^{-1} of *B. toyonensis* (BT2), G_4: fish received diet contained1×10^5 CFU ml^{-1} of *G. stearothermophilus* (GS1) and G_5: fish received diet contained2×10^5 CFU ml^{-1} of *G. stearothermophilus* (GS2).

e_{ij}= the experimental error.

Results

Water quality of the different experimental groups

Dietary treatments had no significant (P>0.05) on pH and nitrites values (Table 2), meanwhile, the other parameters of water quality that includes water temperature, dissolved oxygen, ammonia (NH_4), electric conductivity, total dissolved solids and nitrate (NO_3) were significantly (P<0.05) affected. Fish received diets contained SG2 (G_5) recorded the highest value of water temperature (27.19), electric conductivity (0.592) and Total dissolved solids (453), meanwhile fish that received BT1 (G_2) recorded the highest value of dissolved oxygen (6.25).

Growth performance, specific growth rate, relative growth rate and survival ratio

Results of Table (3) revealed that inclusion probiotic bacteria significantly (P<0.05) increased values of FW, TBWG, ADG, SGR and RGR in comparison with the control. Meanwhile, SR values were improved from 80% to 100% when fish received diets contained probiotic bacteria. Also, mortality rate % improved from 20% in control to Zero % in the other groups that received diets contained probiotic bacteria. The highest values of FW, TBWG, ADG, SGR and RGR recorded when fish received diets contained BT2 (G_3).

Feed utilization

As presented in Table (4)incorporatedprobiotic bacteria caused significantly increasing in values of feed intake (FI), crude protein intake (CPI) and Protein efficiency ratio (PER). In addition to it significantly (P<0.05) improved their values of Feed conversion ratio (FCR) comparing to the control. The best value of FCR recorded when fish receive diet contained BT2 (G_3).

Data presented in Table (5)mentionedthat except for RBC's cell count and Uric acid the other blood parameters had significantly (P<0.05) affected by inclusion probiotic bacteria in fish diets. Values of WBC's cell count, AST and ALT significantly (P<0.05) decreased when fish fed diet contained probiotic bacteria. The lowest value of creatinine (0.325 mg/l) recorded when fish received diet contained SG2 (G_5). Values of total protein, Globulin, hemoglobin concentration, RBC's cell count increased, meanwhile values of uric acid decreased.

Data presented in Table (6)revealed incorporation probiotic bacteria in fish diets had significantly (P<0.05) affecting on all estimated values antioxidants and immune that includes Nitric oxide (NO), Nitro blue tetrazoline (NBT), Lysozyme (LYZO), Catalse enzyme (CAT) and Super oxide dimutase (SOD). On the other hand, main effects of probiotic levels demonstrated that values of NO, NBT, LYZO, CAT and SOD significantly (P<0.05) gradually increased with increasing level of probiotic bacteria introduced compared to the control.

Fish body composition

Data presented in Table (7) mentioned that probiotic bacteria in fish diets had significantly (P<0.05) affecting on all estimated values of fish body composition. Except for values of moisture and dry matter inclusion probiotic bacteria significant (P<0.05) increased values of Organic matter (OM), Crude protein (CP), Ether extract (EE) and Gross energy kcal/ 100g DM or Gross energy cal/ g DM. Meanwhile, values of ash content significantly (P<0.05) decreased in comparison with the control.

Energy retention (ER) and protein productive value (PPV) %

Data of Table (8) cleared that incorporation probiotic bacteria in fish diets significantly (P<0.05) increased values of energy retention (ER) % and protein productive value (PPV) % compared to the control. The corresponding values of ER% were increased by 135.78%, 159.71%, 109.51% and 129.82% for G_2, G_3, G_4 and G_5, respectively in comparison with the control (G_1). Meanwhile values of PPV% were increased by 130.69%, 154.32%, 104.97% and 124.53% for G_2, G_3, G_4 and G_5, respectively in comparison with the control (G_1).

Discussion

The results of this study indicated that dietary supplementation with probiotic strains *Bacillus toyonensis* and *Geobacillusstearothermophilus* had no significant effects on water pH and nitrite (NO_2^-) levels, but significantly influenced other water quality parameters, including temperature, dissolved oxygen (DO), ammonia (NH_4^+), electrical conductivity (EC), total dissolved solids (TDS), and nitrate (NO_3^-). Specifically, fish fed the diet containing *G. stearothermophilus* at 2×10^5 CFU/ml (SG2, G5) showed the highest water temperature (27.19 °C), EC (0.592 mS/cm), and TDS (453 mg/L). Conversely, the highest DO (6.25 mg/L) was observed in fish fed *B. toyonensis* at 1×10^5 CFU/ml (BT1, G2). These findings suggest that the presence of probiotic bacteria may alter microbial activity in the water, possibly enhancing organic matter breakdown and influencing water chemistry. Our results align with those of [43–45], who observed no significant differences in some water quality parameters following probiotic supplementation. Additionally, [44,46] reported that probiotic additions led to a reduction in unionized ammonia (NH_3) concentrations when compared to untreated control groups. This may be due to the nitrifying activity of beneficial bacteria that convert toxic ammonia to less harmful nitrate forms. Similarly, [47] found that waterborne probiotics significantly reduced ammonia concentrations, further supporting

our findings. Meanwhile, [48] observed no significant differences in temperature and DO levels across probiotic treatments, indicating that certain environmental factors may remain stable depending on fish biomass and tank conditions.

Probiotic supplementation significantly improved all assessed growth parameters, including final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) ($P < 0.05$). Survival rate (SR) was also enhanced, rising from 80% in the control group to 100% in treated groups, with zero mortality observed in the latter. The most pronounced improvements were recorded in the BT2 group (G3), which received *B. toyonensis* at 2×10^5 CFU/ml. These results are consistent with [45], who found that dietary probiotics significantly improved FW, TBWG, ADG, SGR, and RGR in Nile tilapia. That study also demonstrated that *G. stearothermophilus* at 1×10^5 CFU/ml (GS1) produced superior growth metrics compared to other groups. The improved performance is likely due to probiotics enhancing nutrient absorption, stimulating appetite, and modulating intestinal microbiota. Similarly, [49,50] reported that diets supplemented with β-glucan, a common prebiotic, increased FW and SGR in fish. However, [51] observed no significant effects of β-glucan on final weight or weight gain, indicating that the effectiveness of such additives may vary with dose, fish species, or experimental conditions.

The present findings showed that dietary supplementation with probiotic bacteria significantly ($P < 0.05$) affected most blood biochemical parameters in Nile tilapia, except for red blood cell (RBC) count and uric acid, which were not significantly altered. Fish fed probiotic-supplemented diets exhibited decreased levels of white blood cells (WBC), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), indicating reduced inflammatory and hepatic stress. The lowest creatinine level (0.325 mg/L) was recorded in fish fed *G. stearothermophilus* at 2×10^5 CFU/ml (SG2, G5), suggesting improved kidney function.

Moreover, levels of total protein, globulin, and hemoglobin increased in probiotic-treated groups, while uric acid levels decreased, reflecting enhanced protein metabolism and reduced nitrogenous waste accumulation.

These findings are partially in agreement with previous studies [49,55], which reported no significant differences in albumin, total protein, or globulin in fish fed prebiotic-supplemented diets. However, the differences in our results may be attributed to the use of probiotics, which have direct effects on the gut microbiota, digestion, and immune responses.

In contrast, [56] found that dietary treatments significantly affected hemoglobin, albumin, total protein, AST, ALT, and creatinine, while WBC, RBC, and urea-N remained unaffected. Additionally, they reported that fish exposed to lead-contaminated diets but supplemented with nano-selenium showed higher hemoglobin, RBC, total protein, and albumin levels than those without supplementation.

Similarly, [57] reported decreased concentrations of WBC, ALT, AST, and creatinine in fish fed functional feed additives. These results support our findings, suggesting that probiotics contribute to reducing metabolic stress and promoting liver and kidney function. Meanwhile, [58] showed that lead contamination in fish diets elevated serum creatinine, ALT, and AST, while reducing total protein and albumin, indicating physiological stress and tissue damage. Furthermore, [59] noted that increased transaminase enzyme levels (ALT and AST) and reduced serum total protein could be linked to protein loss due to kidney dysfunction, reinforcing the interpretation of our results. The inclusion of probiotics in the present study may have prevented such dysfunction. Finally, [60] demonstrated that dietary selenium supplementation enhances liver and kidney health, consistent with our interpretation of improved physiological status in probiotic-fed fish. Overall, the observed improvements in biochemical and hematological indices reinforce the role of dietary probiotics in enhancing fish health and stress resilience. These biomarkers are useful tools for evaluating fish welfare, nutrient assimilation, and the physiological impacts of dietary interventions.

Antioxidants and immune parameters of the different experimental groups

Incorporation of probiotic bacteria into the diet of Nile tilapia resulted in a significant ($P < 0.05$) enhancement of all evaluated antioxidant and immune markers, including nitric oxide (NO), nitro blue tetrazolium (NBT), lysozyme (LYZ), catalase (CAT), and superoxide dismutase (SOD). Furthermore, increasing the probiotic level led to a gradual and significant increase in the values of these parameters compared to the control. These findings are in agreement with previous research [7], which showed that different probiotic strains improved NO levels. However, the same study reported no significant differences in NBT values across treatment groups. Additionally, the concentrations of CAT and SOD increased in all probiotic-treated groups, regardless of the dose used [7].The immune-enhancing role of NO was also highlighted by [61], who described its protective role in viral clearance and immune stimulation. Lysozyme, an important component of innate immunity, was found to be significantly activated in this study, supporting the work of [62], who identified LYZ as a key antimicrobial defense mechanism. Increased LYZ

levels may reflect an enhancement in lytic activity against Gram-positive and Gram-negative bacteria.

Consistently, [63] reported that probiotics significantly increased immune parameters, indicating their immune stimulatory effects. Improved LYZ activity after probiotic treatment was also reported by [64], who noted that commercial probiotics stimulated both LYZ and phagocytic activities. Furthermore, [65] attributed the immune-stimulatory effects of *Bacillus* strains to their peptidoglycan-rich cell walls, which activate host immunity.

Probiotic supplementation significantly ($P < 0.05$) affected the whole-body composition of Nile tilapia. Specifically, values of organic matter (OM), crude protein (CP), ether extract (EE), and gross energy (kcal/100 g DM) increased significantly in probiotic-fed fish, while ash content decreased. No significant changes were observed in moisture and dry matter. These findings are in line with [7], who observed improvements in crude protein and lipid contents across all treatment groups, along with a decrease in ash levels. However, [49] reported no differences in moisture, EE, or ash, and [56] stated that dietary treatments did not significantly affect the body composition of Nile tilapia. Additionally, [66,67] reported an increase in body crude protein content in fish fed β-glucans, which supports the protein-boosting effects observed here. On the contrary, [58] found lead accumulation in fish tissues from contaminated diets, which compromised body composition. Meanwhile, [68] reported no significant changes in moisture, EE, or ash with dietary prebiotics, though [69] found significant differences in crude protein, lipids, ash, and gross energy, indicating variability based on additive type and dose. The present study also demonstrated that dietary probiotic inclusion significantly increased ($P < 0.05$) the energy retention (ER%) and protein productive value (PPV%) compared to the control. ER% increased by 135.78%, 159.71%, 109.51%, and 129.82% for G2, G3, G4, and G5 respectively, while PPV% increased by 130.69%, 154.32%, 104.97%, and 124.53%, respectively.

Similar findings were reported by [70], who showed that adding a bioactive mixture of lemon, onion, and garlic to Nile tilapia diets significantly enhanced both ER and PPV. Their data indicated protein retention improvements of over 120%, which are comparable to our results. In contrast, [71] found that *Saccharomyces cerevisiae* supplementation led to a decrease in ER% but an increase in PPV%, emphasizing that different additives can affect nutrient retention differently.

Additionally, [68] found significant differences in ER% and PPV% among treatments and also noted improvements in economic returns with increased levels of S. cerevisiae in the diet. Likewise, [72] observed that inclusion of black soldier fly larvae meal in fish diets significantly improved ER%, with values increasing by 4.74% to 9.76%, depending on the level of inclusion. However, PPV% was significantly reduced at the highest level (15%) of glycerol monolaurate (GML) inclusion, though fish fed 5% or 10% showed no significant differences compared to control [72].

The improved ER and PPV values observed in this study can be attributed to the enhanced feed efficiency, nutrient digestibility, and protein utilization promoted by probiotic supplementation.

Conclusion

Dietary inclusion of *Bacillus toyonensis* and *Geobacillusstearothermophilus* significantly enhanced growth performance, feed utilization, immune responses, antioxidant activity, and body composition of Nile tilapia. Probiotic supplementation improved protein and energy retention, reduced liver and kidney stress indicators (AST, ALT, creatinine), and boosted immunity (NO, LYZ, CAT, SOD). Higher probiotic levels produced more pronounced effects. These findings support the use of probiotics as effective feed additives for improving fish health, productivity, and sustainability in aquaculture systems.

Acknowledgments

The authors would like to express their sincere appreciation to the laboratory team for their dedicated efforts and valuable contributions, which greatly enhanced the quality and outcomes of this study.

Funding statement

Self-funding

Declaration of Conflict of Interest

No conflict of interest

TABLE 1. Design of the different experimental groups and chemical analysis of the commercial diet

Item	Design of the different experimental groups
G_1 (Control)	Commercial diet
G_2 (BT1)	Commercial diet + 1×10^5 CFU $ml^{-1}$of *B. toyonensis* (BT1)
G_3 (BT2)	Commercial diet + 2×10^5 CFU $ml^{-1}$of *B. toyonensis* (BT2)
G_4 (GS1)	Commercial diet + 1×10^5 CFU $ml^{-1}$of *G. stearothermophilus* (GS1)
G_5 (GS2)	Commercial diet + 2×10^5 CFU $ml^{-1}$of *G. stearothermophilus* (GS2)

Composition of commercial diet

Commercial diet (30% CP) contained the following ingredients that includes:

Soybean meal 48% CP, wheat milling by-product, wheat bran, yellow corn, corn gluten 60% CP, soy oil, NaCl, calcium monohydrate, choline chloride, vitamin & minerals mixture.

Chemical analysis of the commercial diet

Item	%
Moisture	6.88
Dry matter (DM)	93.12
Chemical analysis on dry matter basis	
Organic matter (OM)	87.88
Crude protein (CP)	29.65
Crude fiber (CF)	6.67
Ether extract (EE)	6.23
Ash	12.12
Nitrogen free extract (NFE)	45.33
Gross energy kcal/ 100g dry matter	441.88
Metabolizable energy kcal/ kg DM	342.40
Protein energy ratio (mg CP/ Kcal ME)	86.59

TABLE 2. Effect of incorporation probiotic bacteria on water quality of the different experimental groups.

Item	**Experimental Tilapia fish groups**					**SEM**	**Sign. P<0.05**
	Control	**BT1**	**BT2**	**SG1**	**SG2**		
	G_1	G_2	G_3	G_4	G_5		
Water temperature °C	27.11^{a}	27.16^{a}	26.92^{b}	27.14^{a}	27.19^{a}	0.023	*
Dissolved oxygen (mg/L^{-1})	6.21^{ab}	6.25^{a}	6.19^{ab}	6.22^{ab}	6.16^{b}	0.011	*
pH	7.22	7.20	7.22	7.19	7.17	0.008	NS
Ammonia (NH_4-μM^{-1})	0.006^{a}	0.005^{ab}	0.004^{b}	0.005^{ab}	0.004^{b}	0.0003	*
Electric conductivity (µs/ l)	0.480^{d}	0.485^{d}	0.518^{c}	0.562^{b}	0.592^{a}	0.011	*
Total dissolved solids (µg/ l)	315^{d}	321^{d}	366^{c}	438^{b}	453^{a}	14.075	*
Nitrites (NO_2-μM^{-1})	4.06	4.03	4.01	4.04	4.02	0.010	NS
Nitrate (NO_3-μM^{-1})	8.46^{a}	8.38^{ab}	8.32^{b}	8.41^{ab}	8.35^{ab}	0.018	*

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*

TABLE3. Effect of incorporation probiotic bacteria on growth performance, specific growth rate, relative growth rate and survival ratio of different experimental groups.

Item	Experimental Tilapia fish groups					SEM	Sign. P<0.05
	Control	BT1	BT2	SG1	SG2		
	G_1	G_2	G_3	G_4	G_5		
Number of fish	30	30	30	30	30	-	-
Initial weight, g (IW)	20.41	20.52	20.52	20.47	20.56	0.036	NS
Final weight, g (FW)	44.19^{e}	51.45^{b}	56.27^{a}	47.15^{d}	50.63^{c}	1.047	*
Total body weight gain, g (TBWG)	23.78^{e}	30.93^{b}	35.75^{a}	26.68^{d}	30.07^{c}	1.036	*
Duration experimental period (days)	70 days						
Average daily gain, g (ADG)	0.340^{e}	0.442^{b}	0.511^{a}	0.381^{d}	0.430^{c}	0.015	*
Specific growth rate (SGR)	1.103^{e}	1.312^{b}	1.440^{a}	1.192^{d}	1.288^{c}	0.029	*
Relative growth rate (RGR)	1.165^{e}	1.507^{b}	1.743^{a}	1.304^{d}	1.464^{c}	0.050	*
Number of fish at the starter	30	30	30	30	30	-	-
Number of fish at the end	24	30	30	30	30	-	-
Survival ratio (SR)	80.00^{b}	100^{a}	100^{a}	100^{a}	100^{a}	1.715	*
Number of dead fish	6	00	00	00	00	-	-
Mortality rate percentages	20.00	00	00	00	00	-	-

a, b, c, d and e: Means in the same row having different superscripts differ significantly (P<0.05).
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*

TABLE 4.Effect of incorporation probiotic bacteria on feed utilization of the different experimental groups.

Item	Experimental Tilapia fish groups					SEM	Sign. P<0.05
	Control	BT1	BT2	SG1	SG2		
	G_1	G_2	G_3	G_4	G_5		
Total body weight gain, g (TBWG)	23.78^{e}	30.93^{b}	35.75^{a}	26.68^{d}	30.07^{c}	1.036	*
Feed intake (FI), g	42.43^{c}	44.17^{b}	44.07^{b}	46.70^{a}	45.27^{b}	0.338	*
Feed conversion ratio (FCR)	1.78^{d}	1.43^{b}	1.24^{a}	1.75^{d}	1.51^{c}	0.050	*
Crude protein %				29.65			
Crude protein intake (CPI), g	12.58^{c}	13.10^{b}	13.07^{b}	13.85^{a}	13.42^{b}	0.115	*
Protein efficiency ratio (PER)	1.89^{d}	2.36^{b}	2.74^{a}	1.93^{d}	2.24^{c}	0.076	*

a, b, c, d and e: Means in the same row having different superscripts differ significantly (P<0.05).
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*

Biochemical parameters of different experimental groups

TABLE 5. Effect of incorporation probiotic bacteria on biochemical parameters of different experimental groups.

Item	Experimental Tilapia fish groups					SEM	Sign. P<0.05
	Control	BT1	BT2	SG1	SG2		
	G_1	G_2	G_3	G_4	G_5		
Total protein (g/dl)	4.13^{b}	4.15^{a}	4.17^{a}	4.16^{a}	4.18^{a}	0.006	*
Albumin (g/dl)	2.23^{a}	2.24^{a}	2.23^{a}	2.18^{b}	2.22^{a}	0.006	*
Globulin (g/dl)	1.90^{d}	1.91^{cd}	1.94^{bc}	1.98^{a}	1.96^{ab}	0.008	*
Albumin: Globulin ratio	1.17^{a}	1.17^{a}	1.15^{ab}	1.10^{c}	1.13^{bc}	0.007	*
Hemoglobin concentration (g/dl)[1]	11.83^{c}	11.88^{abc}	11.93^{a}	11.86^{bc}	11.90^{ab}	0.011	*
RBC's cell count ($x10^6$/µl) [2]	1.66	1.69	1.72	1.68	1.70	0.009	NS
WBC's cell count ($x10^6$/µl) [3]	4.84^{a}	4.76^{b}	4.73^{b}	4.75^{b}	4.71^{b}	0.014	*
Liver function							
AST (Unit/l)	22.18^{a}	21.90^{bc}	21.85^{c}	21.94^{b}	21.88^{bc}	0.034	*
ALT (Unit/l)	9.75^{a}	9.62^{b}	9.43^{c}	9.66^{b}	9.48^{c}	0.031	*
Kidneys function							
Uric acid (mg/l)	1.76	1.72	1.69	1.73	1.70	0.010	NS
Creatinine (mg/l)	0.333^{a}	0.330^{ab}	0.326^{ab}	0.329^{ab}	0.325^{b}	0.001	*

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).
AST: Aspartate aminotransferase. ALT: Alanine aminotransferase.
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*
Antioxidants and immune parameters of the different experimental groups

TABLE 6. Effect of incorporation probiotic bacteria on antioxidants and immune parameters of the different experimental groups.

Item	Experimental Tilapia fish groups					SEM	Sign. P<0.05
	Control	BT1	BT2	SG1	SG2		
	G_1	G_2	G_3	G_4	G_5		
Nitric oxide (NO)	0.329^{d}	0.755^{c}	0.900^{a}	0.850^{a}	0.935^{a}	0.062	*
Nitro blue tetrazoline (NBT)	0.536^{c}	0.768^{ab}	0.799^{ab}	0.884^{a}	0.680^{bc}	0.037	*
Lysozyme (LYZO)	0.439^{e}	0.551^{d}	0.811^{b}	0.743^{c}	0.962^{a}	0.048	*
Catalse enzyme (CAT)	29.25^{e}	64.50^{b}	56.50^{c}	49.00^{d}	70.00^{a}	3.864	*
Super oxide dimutase (SOD)	15.50^{c}	24.17^{b}	28.33^{ab}	26.50^{ab}	30.50^{a}	1.515	*

a, b, c, d and e: Means in the same row having different superscripts differ significantly (P<0.05).
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*

TABLE 7. Effect of incorporation probiotic bacteria on fish body composition of the different experimental groups.

Item	Initial fish body composition	Experimental Tilapia fish groups					SEM	Sign. P<0.05
		Control	BT1	BT2	SG1	SG2		
		G_1	G_2	G_3	G_4	G_5		
Moisture	77.43	75.57^{a}	75.27^{ab}	74.84^{b}	75.58^{a}	75.64^{a}	0.089	*
Dry matter (DM)	22.57	24.43^{b}	24.73^{ab}	25.16^{a}	24.42^{b}	24.36^{b}	0.089	*
Chemical analysis onDM basis		*Chemical analysis on DM basis*						
Organic matter (OM)	77.98	81.85^{e}	86.12^{c}	87.54^{a}	84.98^{d}	86.39^{b}	0.536	*
Crude protein (CP)	59.53	62.42^{d}	64.57^{b}	65.68^{a}	63.66^{c}	64.66^{b}	0.297	*
Ether extract (EE)	18.45	19.43^{b}	21.55^{a}	21.86^{a}	21.32^{a}	21.73^{a}	0.264	*
Ash	22.02	18.15^{a}	13.88^{c}	12.46^{e}	15.02^{b}	13.61^{d}	0.536	*
Gross energy kcal/100g DM	509.77	535.32^{d}	567.39^{b}	576.58^{a}	560.09^{c}	569.59^{b}	3.978	*
Gross energy cal/ g DM	5.0977	5.3532^{d}	5.6739^{b}	5.7658^{a}	5.6009^{c}	5.6959^{b}	0.040	*

a, b, c, d and e: Means in the same row having different superscripts differ significantly (P<0.05).
SEM: Standard error of the mean. *: Significant at (P<0.05).
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*

TABLE 8. Effect of incorporation probiotic bacteria on *energy retention (ER) %* and *protein productive value (PPV) %*of different experimental groups.

Item	Experimental Tilapia fish groups					SEM	Sign. P<0.05
	Control	BT1	BT2	SG1	SG2		
	G_1	G_2	G_3	G_4	G_5		
Initial weight (IW), g	20.41	20.52	20.52	20.47	20.56	0.036	NS
Final weight (FW), g	44.19^{e}	51.45^{b}	56.27^{a}	47.15^{d}	50.63^{c}	1.047	*
Calculation the energy retention (ER) %							
Energy content in final body fish (cal / g)	5.3532^{d}	5.6739^{b}	5.7658^{a}	5.6009^{c}	5.6959^{b}	0.340	*
Total energy at the end in body fish (E)	236.56^{d}	291.92^{b}	324.44^{a}	264.08^{c}	288.38^{b}	7.664	*
Energy content in initial body fish (cal / g)	5.0977						
Total energy at the start in body fish (E_0)	104.04	104.60	104.60	104.35	104.81	0.186	NS
Energy retained in body fish ($E-E_0$)	132.52^{d}	187.32^{b}	219.84^{a}	159.73^{c}	183.57^{b}	7.605	*
Energy of the feed intake (Cal / g feed)	4.419						
Quantity of feed intake	42.43^{c}	44.17^{b}	44.07^{b}	46.70^{a}	45.27^{b}	0.388	*
Total energy of feed intake (EF)	187.50^{c}	195.19^{b}	194.75^{b}	206.37^{a}	200.05^{b}	1.714	*
Energy retention (ER) %	70.68^{e}	95.97^{b}	112.88^{a}	77.40^{d}	91.76^{c}	3.707	*
Calculation the protein productive value (PPV) %							
Crude protein % in final body fish	62.42^{d}	64.57^{b}	65.68^{a}	63.66^{c}	64.66^{b}	0.297	*
Total protein at the end in body fish (PR_1)	27.58^{d}	33.22^{b}	36.96^{a}	30.02^{c}	32.74^{b}	0.814	*
Crude protein % in initial body fish	59.53						
Total protein at the start in body fish (PR_2)	12.15	12.22	12.22	12.19	12.24	0.022	NS
Protein retained in body fish (PR_3)	15.43^{d}	21.00^{b}	24.74^{a}	17.83^{c}	20.50^{b}	0.807	*
Crude protein in feed intake (CP %)	29.65						
Total Protein intake (PI), g	12.58^{c}	13.10^{b}	13.07^{b}	13.85^{a}	13.42^{b}	0.115	*
Protein productive value (PPV) %	122.66^{e}	160.31^{b}	189.29^{a}	128.74^{d}	152.76^{c}	5.881	*

a, b, c, d and e: Means in the same row having different superscripts differ significantly (P<0.05).
$PR_3 = PR_1 – PR_2$.SEM: Standard error of the mean. NS: Not significant *: Significant at (P<0.05).
BT1: group fish received diet contained 1×10^5 CFU ml^{-1} *of B. toyonensis.*
BT2: group fish group received diet contained 2×10^5 CFU ml^{-1} *of B. toyonensis.*
GS1: group fish received diet contained 1×10^5 CFU ml^{-1} *of G. stearothermophilu.*
GS2: group fish received diet contained 2×10^5 CFU ml^{-1} *of G. stearothermophilu.*

References

1. Pepi, M. and Focardi, S. Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the mediterranean area. *International Journal of Environmental Research and Public Health*, **18**, (2021).doi:10.3390/ijerph18115723.
2. El-Kady, A.A.,Magouz, F.I., Mahmoud, S.A. and Abdel-Rahim, M.M. The effects of some commercial probiotics as water additive on water quality, fish performance, blood biochemical parameters, expression of growth and immune-related genes, and histology of Nile tilapia (Oreochromis niloticus). *Aquaculture*, **546**, 737249 (2022),
3. Naiel, M.A.,Gewida, A.G., Merwad, A.-R.M., Abdel-Hamid, E.A., Negm, S.S., Alagawany, M. and Farag, M.R. The effects of various organic fertilizers with or without adsorbents on the productivity, antioxidant status and immune responses of Nile tilapia raised in cement ponds. *Aquaculture*, **548**, 737593 (2022).
4. Naiel, M.A.,Farag, M.R., Gewida, A.G., Elnakeeb, M.A., Amer, M.S. and Alagawany, M. Using lactic acid bacteria as an immunostimulants in cultured shrimp with special reference to Lactobacillus spp. *Aquaculture International*, **29**, 219-231 (2021).
5. Jahangiri, L. and Esteban, M.Á. Administration of probiotics in the water in finfish aquaculture systems: a review. *Fishes*, **3**, 33 (2018).

6. Adorian, T.J.,Jamali, H., Farsani, H.G., Darvishi, P., Hasanpour, S., Bagheri, T. and Roozbehfar, R. 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**, 248-255 (2019).

7. Naiel, M.A.,Shehata, A.M., El-Kholy, A.I., El-Naggar, K., Farag, M.R. and Alagawany, M. The mitigating role of probiotics against the adverse effects of suboptimal temperature in farmed fish: a review. *Aquaculture*, 550, 737877 (2022).

8. Reda, R.M. and Selim, K.M. Evaluation of Bacillus amyloliquefaciens on the growth performance, intestinal morphology, hematology and body composition of Nile tilapia, Oreochromis niloticus. *Aquaculture International*, **23**, 203-217 (2015).

9. Sutthi, N., Thaimuangphol, W., Rodmongkoldee, M., Leelapatra, W. and Panase, P. Growth performances, survival rate, and biochemical parameters of Nile tilapia (*Oreochromis niloticus*) reared in water treated with probiotic. *Comparative Clinical Pathology*, **27**, 597-603 (2018).

10. Zaineldin, A.I.,Hegazi, S., Koshio, S., Ishikawa, M., Bakr, A., El-Keredy, A.M.S., Dawood, M.A.O., Dossou, S., Wang, W. and Yukun, Z. *Bacillus subtilis* as probiotic candidate for red sea bream: Growth performance, oxidative status, and immune response traits. *Fish &Shellfish Immunology*, **79**, 303-312 (2018). doi:10.1016/j.fsi.2018.05.035.

11. Zhou, X., Tian, Z., Wang, Y. and Li, W. Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Fish Physiology and Biochemistry*, **36**, 501-509 (2010). doi:10.1007/s10695-009-9320-z

12. Chatterjee, S.K.,Bhattacharjee, I. and Chandra, G. Biosorption of heavy metals from industrial waste water by *Geobacillus thermodenitrificans*. *Journal of Hazardous Materials*, **175**, 117-125 (2010). doi:10.1016/j.jhazmat.2009.09.136.

13. Hlordzi, V., Kuebutornye, F.K., Afriyie, G., Abarike, E.D., Lu, Y., Chi, S. and Anokyewaa, M. The use of *Bacillus* species in maintenance of water quality in aquaculture: A review. *Aquaculture Reports*, **18**, 100503 (2020).

14. Nikoskelainen, S., Ouwehand, A., Salminen, S.and Bylund, G. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. *Aquaculture*, **198**, 229-236 (2001).

15. V.H. Bernard, A.N., M.Y. Ina-Salwany and Yasser Abdelhadi. *Bacillus cereus*, JAQ04 Strain as a potential probiotic for red tilapia, *Oreochromis*species. *Asian Journal of Animal and Veterinary Advances*, **8**, 395-400 (2013).

16. *AOAC Association of Official Analytical Chemists (2016) Official Methods of Analysis of International.*

17. Armstrong, W.D. and Carr, C.W. *Physiological chemistry: Laboratory directions*, Burgess: 1963.

18. Cannon, D., Olitzky, I. and Inkpen, J. Proteins In: Clinical chemistry, principles and techniques. 1974.

19. Witt, D. and Trendelenburg, C. Quatitative determination of CK-MB in human serum and plasma. *Clin. Bioch.*, **20**, 235 (1982).

20. Doumas, B.T., Watson, W.A. and Biggs, H.G. Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*, **31**, 87-96 (1971).

21. Tietz, N. A method for the rapid determination of albumin of blood plasma.*textbook of clinical chemistry. WB Saunders Company, Philadelphia* 1986, 589.

22. Tietz, N.W. Clinical guide to laboratory tests. In *Clinical guide to laboratory tests*, pp. 1096-1096 (1995).

23. Reda, F.M., Madkour, M., El-Azeem, N.A., Aboelazab, O., Ahmed, S.Y.A. and Alagawany, M. Tomato pomace as a nontraditional feedstuff: productive and reproductive performance, digestive enzymes, blood metabolites, and the deposition of carotenoids into egg yolk in quail breeders. *Poultry Science*, **101**, 101730(2022). doi:10.1016/j.psj.2022.101730.

24. Alagawany, M., El-Saadony, M.T., El-Rayes, T.K., Madkour, M., Loschi, A.R., Di Cerbo, A. and Reda, F.M. Evaluation of dried tomato pomace as a non-conventional feed: Its effect on growth, nutrients digestibility, digestive enzyme, blood chemistry and intestinal microbiota of growing quails. *Food and Energy Security*,**11**, e373. (2022).

25. Gupta, S.K., Pal, A.K., Sahu, N.P., Dalvi, R., Kumar, V. and Mukherjee, S.C. Microbial levan in the diet of Labeo rohita Hamilton juveniles: effect on non-specific immunity and histopathological changes after challenge with Aeromonas hydrophila. *Journal of Fish Diseases*, **31**, 649-657(2008). doi:10.1111/j.1365-2761.2008.00939.x.

26. Goldman, L. and Schafer, A.I. *Goldman's cecil medicine E-book*, Elsevier Health Sciences: 2011.

27. McPherson, R.A. and Pincus, M.R. Henry's clinical diagnosis and management by laboratory methods. *PhiladelphiaPA: Saunders* 2011.

28. Weiss, D.J., Wardrop, K.J. *Schalm's veterinary hematology*, John Wiley & Sons: 2011.

29. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*,,**28**, 56-63(1957). doi:10.1093/ajcp/28.1.56.

30. Harold, V. Colorimetric determination of glutamate pyruvate and oxaloacetic transaminase, *Practical Clin. Biochem. 4th Ed.: P* 1975, *294*.

31. El-Wardany, I., Shourrap, M., Madkour, M. and Abd El-Azeem, N.A. Effect of Age at mating and silver nanoparticles administration on progeny productive performance and some blood constituents in Japanese quail. *International Journal of Chem.Tech. Research*, **9**, 21-34 (2016).

32. Nishikimi, M., Appaji, N. and Yagi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, **46**, 849-854(1972).doi:10.1016/s0006-291x(72)80218-3.

33. Aebi, H. Catalase in vitro. *Methods in Enzymology* **105**, 121-126 (1984). doi:10.1016/s0076-6879(84)05016-3.

34. Madkour, M., Alaqaly, A.M., Soliman, S.S., Ali, S.I. and Aboelazab, O. Growth performance, blood biochemistry, and mRNA expression of hepatic heat shock proteins of heat-stressed broilers in response to rosemary and oregano extracts. *Journal of Thermal Biology*, **119**, 103791 (2024). doi:10.1016/j.jtherbio.2024.103791.

35. Rajaraman, V., Nonnecke, B.J., Franklin, S.T., Hammell, D.C. and Horst, R.L. Effect of vitamins A and E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer. *Journal of Dairy Science*, **81**, 3278-3285(1998) doi:10.3168/jds.S0022-0302(98)75892-8.

36. Siwicki, A., Studnicka, M. and Ryka, B. Phagocytic ability of neutrophils in carp (cyprinus-carpio l). *Bamidgeh.*, **37**, 123-128 (1985).

37. Schaperclaus, W., Kulow, H. and Schrenkenbach, K. Infections abdominal dropsy. İn Schaperclaus, W (Eds). . 1992.

38. Blaxter, K.L. The energy metabolism of ruminants. 1962.

39. MacRae, J. and Lobley, G. Some factors which influence thermal energy losses during the metabolism of ruminants. *Livestock Production Science*, **9**, 447-456(1982).

40. NRC. *NRC, 1994*, National Academies Press: 1994.

41. Salcedo, J., McCormick, K. *SPSS statistics for dummies*, John Wiley & Sons: 2020.

42. Duncan, D.B. Multiple range and multiple F tests. *Biometrics*, **11**, 1-42(1955).

43. Correia, E.S., Wilkenfeld, J.S., Morris, T.C., Wei, L., Prangnell, D.I. and Samocha, T.M. Intensive nursery production of the Pacific white shrimp Litopenaeus vannamei using two commercial feeds with high and low protein content in a biofloc-dominated system. *Aquacultural Engineering*, **59**, 48-54(2014).

44. Khanjani, M.H., Alizadeh, M., Mohammadi, M. and Sarsangi Aliabad, H. Biofloc system applied to Nile tilapia (Oreochromis niloticus) farming using different carbon sources: Growth performance, carcass analysis, digestive and hepatic enzyme activity. *Iranian Journal of Fisheries Sciences*, **20**, 490-513(2021).

45. Naiel, M.A., Abdelghany, M.F., Khames, D.K., Abd El-hameed, S.A., Mansour, E.M., El-Nadi, A.S. and Shoukry, A.A. Administration of some probiotic strains in the rearing water enhances the water quality, performance, body chemical analysis, antioxidant and immune responses of Nile tilapia, Oreochromis niloticus. *Applied Water Science*, **12**, 209(2022).

46. Tahoun, A. Addition of commercial probiotic (Lactobacillus delbruekii and L. fermentum) in red tilapia broodstock diet in different rearing systems. I-effects on reproductive performance and larval quality. *Egyptian Journal of Nutrition Feeds*, **25**, 123-134(2022).

47. John, E.M., Krishnapriya, K. and Sankar, T. Treatment of ammonia and nitrite in aquaculture wastewater by an assembled bacterial consortium. *Aquaculture*, **526**, 735390(2020).

48. Eid, A., Showhdy, K.G., Ali, M.A. and Elfeky, A. Effect of reuse biofloc water on growth performance, feed utilization of Nile tilapia fingerlings (Oreochromis niloticus). *Egyptian Journal of Nutrition Feeds*, **24**, 315-326(2021).

49. Ali, B.A. and El-Feky, A. Enhancing growth performance and feed utilization using prebiotics in commercial diets of Nile Tilapia (Oreochromis niloticus) fingerlings. *Egyptian Journal of Nutrition Feeds*,**22**, 219-225(2019).

50. López, N., Cuzon, G., Gaxiola, G., Taboada, G., Valenzuela, M., Pascual, C., Sánchez, A.and Rosas, C. Physiological, nutritional, and immunological role of dietary β 1-3 glucan and ascorbic acid 2-monophosphate in Litopenaeus vannamei juveniles. *Aquaculture*, **224**, 223-243 (2003).

51. Nieves-Rodríguez, K.N., Álvarez-González, C.A., Peña-Marín, E.S., Vega-Villasante, F., Martínez-García, R., Camarillo-Coop, S., Tovar-Ramírez, D., Guzmán-Villanueva, L.T., Andree, K.B. and Gisbert, E. Effect of β-glucans in diets on growth, survival, digestive enzyme activity, and immune system and intestinal barrier gene expression for tropical gar (Atractosteus tropicus) juveniles. *Fishes*, **3**, 27(2018).

52. Dalmo, R.A. and Bøgwald, J. Beta-glucans as conductors of immune symphonies. *Fish &Shellfish Immunology*, **25**, 384-396(2008). doi:10.1016/j.fsi.2008.04.008.

53. Talpur, A.D., Munir, M.B., Mary, A. and Hashim, R. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against Aeromonas hydrophila in snakehead (Channa striata) fingerlings. *Aquaculture*, **426**, 14-20 (2014).

54. Hoseinifar, S.H., Safari, R. and Dadar, M. Dietary sodium propionate affects mucosal immune parameters, growth and appetite related genes expression: Insights from zebrafish model. *General and Comparative Endocrinology*, **243**, 78-83(2017). doi:10.1016/j.ygcen.2016.11.008.

55. Guerreiro, I., Couto, A., Machado, M., Castro, C., Pousão-Ferreira, P., Oliva-Teles, A. and Enes, P. Prebiotics effect on immune and hepatic oxidative status and gut morphology of white sea bream (Diplodus sargus). *Fish &Shellfish Immunology*, **50**, 168-174(2016). doi:10.1016/j.fsi.2016.01.023.

56. Ahmed, N.H., Ayyat, A., Abdelrhman, A. and Abd El-Latif, K. The ameliorative effect of nano-selenium supplementation on growth, body composition, lead bioaccumulation, and blood components of nile tilapia

fed lead-contaminated diet. *Egyptian Journal of Nutrition Feeds*, **26**, 109-118(2023).

57. Peres, H., Santos, S. and Oliva-Teles, A. Selected plasma biochemistry parameters in gilthead seabream (S parus aurata) juveniles. *Journal of Applied Ichthyology*, **29**, 630-636 (2013).

58. Ayyat, M.S., Ayyat, A.M.N., Abd El-Latif, K.M., Hessein, A.A.A. and Al-Sagheer, A.A. Inorganic mercury and dietary safe feed additives enriched diet impacts on growth, immunity, tissue bioaccumulation, and disease resistance in Nile tilapia (Oreochromis niloticus). *Aquatic Toxicology (Amsterdam, Netherlands)* **224**, 105494(2020), doi:10.1016/j.aquatox.2020.105494.

59. Ayyat, M.S., Ayyat, A.M., Naiel, M.A. and Al-Sagheer, A.A. Reversal effects of some safe dietary supplements on lead contaminated diet induced impaired growth and associated parameters in Nile tilapia. *Aquaculture*, **515**, 734580 (2020).

60. Özkan-Yilmaz, F., Özlüer-Hunt, A., Gündüz, S.G., Berköz, M. and Yalin, S. Effects of dietary selenium of organic form against lead toxicity on the antioxidant system in Cyprinus carpio. *Fish Physiology and Biochemistry*, **40**, 355-363(2014).doi:10.1007/s10695-013-9848-9.

61. Eddy, F.B. Role of nitric oxide in larval and juvenile fish. *Comparative biochemistry and physiology. Part A, Molecular &Integrative Physiology*, **142**, 221-230(2005). doi:10.1016/j.cbpb.2005.05.038.

62. Li, L., Cardoso, J.C.R., Félix, R.C., Mateus, A.P., Canário, A.V.M. and Power, D.M. Fish lysozyme gene family evolution and divergent function in early development. *Developmental and Comparative Immunology*, **114**, 103772 (2021). doi:10.1016/j.dci.2020.103772.

63. Kord, M.I., Maulu, S., Srour, T.M., Omar, E.A., Farag, A.A., Nour, A.A.M., Hasimuna, O.J., Abdel-Tawwab, M. and Khalil, H.S. Impacts of water additives on water quality, production efficiency, intestinal morphology, gut microbiota, and immunological responses of Nile tilapia fingerlings under a zero-water-exchange system. *Aquaculture*, **547**, 737503. (2022).

64. Höltje, J.V. Bacterial lysozymes. *Exs.*,**75**, 65-74 (1996). doi:10.1007/978-3-0348-9225-4_4.

65. Tocheva, E.I., López-Garrido, J., Hughes, H.V., Fredlund, J., Kuru, E., Vannieuwenhze, M.S., Brun, Y.V., Pogliano, K. and Jensen, G.J. Peptidoglycan transformations during Bacillus subtilis sporulation. *Molecular Microbiology*, **88**, 673-686(2013). doi:10.1111/mmi.12201.

66. Eleraky, W. and Reda, R. Evaluation of prebiotic and probiotic dietary supplementation on growth performance and some blood parameters of Cyprinus carpio Frys. *Egyptian Journal of Aquatic Biology and Fisheries*, **18**, 29-38 (2014).

67. Hassaan, M.S., Mahmoud, S.A., Jarmolowicz, S., El-Haroun, E.R., Mohammady, E.Y. and Opiyo, E. Climate-resilience of rural chicken is in the genes [Internet]. *Sci. Dev. Net*., 18023 (2021). https://hdl.handle.net/10568/118023

68. Davies, S.J.J.A.N. Effects of dietary baker's yeast extract on the growth, blood indices and histology of Nile tilapia (Oreochromis niloticus L.) fingerlings. *Aquaculture Nutrition*, **24**, 1709-1717(2018).

69. Mabroke, R.S., Zidan, A.E.-N.F., Tahoun, A.-A., Mola, H.R., Abo-State, H. and Suloma, A.J.A.R. Feeding frequency affect feed utilization of tilapia under biofloc system condition during nursery phase., *Aquaculture Reports*, **19**(5),100625(2021).

70. El-Dakar, A., Mouhmed, A., Elgamal, A., Amer, M., Shalaby, S. and Abdel-Aziz, M.F.A. Effect of re-feeding regime under different stocking density of Nile tilapia, O. niloticus on growth performance, nutritional efficiency and fish body composition. *Mediterranean Aquaculture Journal*,**10**, 1-9(2023).

71. Abozaid, H., Elnadi, A., Mansour, H., Aboelhassan, D.M., Awad, E., El- Nomeary, Y., Omar, H.A., Abbas, W.T. and Farag, I.M. Nutritional Effect of Using a Bioactive Mixture (Lemon, Onion and Garlic) on Growth Performances, Feed Utilization, Immune Status and Gene Expression of Nile tilapia (Oreochromis niloticus). *Egyptian Journal of Veterinary Sciences*, **55**, 1669-1684(2024).

72. Abozaid, H., Elnadi, A.S., Aboelhassan, D.M., El-Nomeary, Y.A., Omar, H.A. and Abbas, W.T.J.E.J.o.A.B. Using the Dried Yeast (Saccharomy cescerevisiae) as a Growth Promoter in the Nile Tilapia (Oreochromis niloticus) Diets. *Egyptian Journal of Aquatic Biology and Fisheries*, **28**(2),34 (2024).

73. Fayed, W.M., Mansour, A.T., Zaki, M.A., Omar, E.A., Nour, A.M., Taha, E.M. and Sallam, G.R. Water quality change, growth performance, health status in response to dietary inclusion of black soldier fly larvae meal in the diet of Nile tilapia, Oreochromis niloticus. *Annals of Animal Science*, **24**, 533-544(2024).

الأثر الغذائي لإضافة بكتيريا البروبيوتيك في علائق زريعة أسماك البلطي النيلي (*Oreochromis niloticus*)

دعاء خميس[1]، علي النادي[2]، حامد عمر[2] و سماح عبد الحميد[1]

[1] قسم تغذية الأسماك وتقنيات الأعلاف، المعمل المركزي لبحوث الاستزراع السمكي(CLAR) ، العباسة، أبو حماد ٤٤٦٦٢، مركزالبحوث الزراعية،الجيزة،مصر.
[2] قسم الإنتاج الحيواني، معهد بحوث الزراعة الحيوية، المركز القومي للبحوث، الدقي، الجيزة ١٢٦٢٢، مصر.

الملخص

تم استخدام عدد إجمالي ١٥٠ سمكه من زريعة أسماك البلطي النيلي بمتوسط وزن ابتدائي ٢٠,٤٨ جرام، لدراسة تأثير إضافة بكتيريا البروبيوتيك في علائق الأسماك على أدائها، وكفاءة استخدام العلف، ومقاييس الدم، وتركيب الجسم، و الطاقة(ER) ، والقيمة الإنتاجية للبروتين .%(PPV) تم توزيع الأسماك على ١٥ حوضًا زجاجيًا، وُضع في كل حوض ١٠ أسماك بسعة ١٠٠ لتر. تم إدخال سلالتين من البروبيوتيك *Bacillus toyonensis*) و *Geobacillus* بتركيزين ١×10^5 و٢×10^5 وحدة مكونة للمستعمرات/(CFU) مل إلى العليقة مقارنةً بالمعاملة الضابطة. أظهرت النتائج أن المعاملات الغذائية أثرت بشكل معنوي على جودة المياه، بما في ذلك درجة الحرارة، والأوكسجين المذاب، والأمونيا (NH_4)، والتوصيل الكهربائي، والمواد الصلبة الذائبة الكلية، والنترات. كما سجلت قيم الوزن النهائي(FW) ، والزيادة الوزنية الكلية (TBWG) ، ومتوسط الزيادة اليومية(ADG) ، ومعدل النمو النوعي (SGR) ، ومعدل النمو النسبي (RGR) زيادات معنوية، وارتفعت نسبة البقاء (SR) من ٨٠% إلى ١٠٠%، بينما انخفضت نسبة النفوق من ٢٠% في المعاملة الضابطة إلى ٠% مع استخدام البروبيوتيك. تاثرت المعاملات الغذائية بشكل معنوي ومعامل التحويل الغذائي .(FCR) وانخفضت أعداد كريات الدم البيضاء، وإنزيمي AST وALT، ومستوى حمض اليوريك، بينما ارتفعت مستويات البروتين الكلي، والجلوبين، وتركيز الهيموجلوبين، وعدد كريات الدم الحمراء. كذلك، زادت تدريجيًا وبصورة معنوية مستويات أكسيد النيتريك(NO) ، والتترازولين (NBT)، والليزوزيم (LYZO) ، وإنزيم الكاتاليز (CAT)، وإنزيم السوبر أوكسيد ديسميوتاز (SOD) مع زيادة تركيز البروبيوتيك كما أدت إضافة البروبيوتيك إلى زيادة معنوية في محتوى المادة العضوية، والبروتين الخام، والدهن الخام، والطاقة الكلية، مع انخفاض في محتوى الرماد. وسجلت زيادات معنوية في كل من ER% و .%PPVوتُشير النتائج إلى أن إضافة بكتيريا البروبيوتيك في علائق الأسماك يُحسّن من أداء النمو، وكفاءة استخدام العلف، ومعايير الدم، وتركيب الجسم، الاستفاده من الطاقة، والقيمة الإنتاجية للبروتين، دون أي آثار سلبية على الأسماك.

الكلمات داله : تغذية الاستزراع السمكي، أداء النمو، المؤشرات الدموية، النشاط الإنزيمي، ميكروبيوم الأمعاء، الحالة الصحية، معدل البقاء، تركيب الجسم، كفاءة استخدام العلف.