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Effects of probiotics dietary supplementation on growth performance, feed utilization, and physiological responses in Nile tilapia fingerlings

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Abstract. *Mohammed FA, Yousif RA, Alnoor NA, Suliman SM. 2026. Effects of probiotics dietary supplementation on growth performance, feed utilization, and physiological responses in Nile tilapia fingerlings. Asian J Trop Biotechnol 23 (1): c230101. https://doi.org/10.13057/biotek/c230101.* This study was conducted to evaluate the effect of different dietary probiotics groups on water quality, growth performance, feed utilization and hematological parameters for Nile tilapia (*Oreochromis niloticus*). A seven-week feeding trial was conducted to evaluate growth, feed utilization and body composition of Nile tilapia fingerlings *O. niloticus* (1.74±0.02 g) fed five isonitrogenous (30.2% crude protein) and isoenergetic (4382.76 Kcal/kg/gross energy) diets, however, the control diet (D1) had no probiotic supplement. Diets 2-5 were formulated to be D2 (*Bacillus subtilis* NIOFSD017, 10^7 CFU/g) D3 (*Lactobacillus plantarum* NIOFSD018, 10^7 CFU/g), D4 (mixture of NIOFSD017, 0.5×10^7 CFU/g and NIOFSD018, 0.5×10^7 CFU/g) and D5 (*Saccharomyces cerevisiae* NIOFSD019, 10^4 CFU/g). The experimental design was completely randomized with five treatments and three replicates. Fish were stocked in triplicate groups of 20 fish. Fish fed diet 5 (D5) exhibited the highest ($P<0.05$) values for live weight gain and specific growth rate (% per day). FCR and SGR were better ($P<0.05$) in fish-fed diets 1 (D1) and (D2). Fish-fed diet D1 exhibited lower ($P<0.05$) fat and ash contents in carcass. The protein efficiency ratio, absolute body weight live weight gain and feed conversion ratio content significantly decreased in fish fed diet D1 (no probiotic supplement).

Keywords: Aquaculture nutrition, feed utilization, growth performance, Nile tilapia, probiotic

INTRODUCTION

Probiotics have increasingly been integrated into Nile tilapia aquaculture as sustainable bio-additives to enhance fish performance, health, immune responses, and culture conditions. Recent research highlights multiple benefits of probiotic supplementation, including improved growth performance, feed utilization, intestinal health, immune function, and resistance to pathogens. Aquaculture is considered to be the answer to the current shortage of commercial fishery production, and its intensification has led to the reliability of artificial feeding (Noori 2013; Zhang et al. 2014; Dawood et al. 2015; Limbu 2019; Zehra and Yousif 2021; Yousif et al. 2022). Tilapia has become the most prominent fish species in the world and plays a growing role in the global aquaculture trade. Tilapia is a freshwater fish belonging to the Cichlidae family. It is currently recognized as the aquatic chicken owing to its rapid growth, adaptability to disease, high meat quality, capability to grow and reproduce in captivity and feed at low trophic levels (Nguyen 2007; Bhujel 2014; Chirapongsatonkul et al. 2019). Tilapia is currently cultivated in more than 100 countries worldwide (El-Sayed 2006; Bhujel et al. 2007; Bhujel 2014; FAO 2017). Tilapia is farmed much faster and on a larger scale than any other group of fish. Rapid advances in livestock and a wide range of uses could mean that tilapia may eventually overtake carp to become the most important domestic fish. Tilapia has already become one of the most important farmed fish and is increasingly involved in international maritime trade (Fitzsimmons 2006; Prabu et al. 2019). Feed represents a major cost for intensive tilapia production and it is one of the most important factors that influence the ability of fish to attain their genetic growth potential and maintain proper health. Research on nutrition and feeding of tilapia has been expanded steadily over the past three decades, including the use of potential new functional ingredients, feed additives and probiotics to improve the growth, feed utilization and fish health (Deng et al. 2015; Abeer and El-Wahab 2019; Mohammed et al. 2020; Zehra et al. 2023). Probiotics are live microorganisms, which have beneficial effects on the host by modifying the host-associated or ambient microbial community of the gastrointestinal tract, thus promoting better feed utilization, enhancing the host response towards disease and improving the quality of its ambient environment (Verschuere et al. 2000; Devic et al. 2018; Fachri et al. 2024). Although the importance of probiotics in human and animal nutrition is widely recognized (Rinkinen et al. 2003), in recent years, the role of probiotics in nutrition and health of certain aquaculture species has also been investigated and subject to reviews (Merrifield et al. 2010). It appears that probiotics provide benefits by establishing favorable microbial communities such as lactic acid bacteria and *Bacillus* sp. in the gastrointestinal track which may alter gut morphology and produce certain enzymes and inhibitory compounds, causing improved digestion and absorption of nutrients as well as enhanced immune response (Verschuere et al.

2000; Tayyab et al. 2025). Several studies have demonstrated that the use of probiotics improves the health of larval and juvenile fish, disease resistance, growth performance and body composition; however, the mode of action in fish species may vary between farmed fish species cultured in freshwater and marine environments.

The use of probiotics in feeds to improve growth of different fish species, including African catfish, *Clarias gariepinus*; Senegalese sole, *Solea senegalensis*, tilapia, *Oreochromis niloticus* (El-Haroun et al. 2006), has been investigated. The effects of probiotics have been linked to modulation of gut microbiota and establishment of the beneficial microorganisms, higher specific and total digestive enzyme activities in the brush-border membrane, which increases the nutrient digestibility and feed utilization (Kesarcodi et al. 2008). In addition, the production of vitamins by these gut microbes could also increase vitamin synthesis and improve fish health. Endogenous digestive enzymes in fish have been studied by several workers (Chan et al. 2008; Fachri et al. 2024).

However, information regarding the enzyme-producing intestinal bacteria, their source and their effect on fish digestion and metabolism is scarce. So, the present study was designed to evaluate the effect of different dietary probiotics groups (*Bacillus subtilis*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*) on water quality, growth performance, feed utilization and hematological parameters of Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

Study area

The experimental study was conducted in Sudan University of Science and Technology, College of Animal Production Science and Technology, at the Wet Laboratory of the Department of Fisheries and Wildlife Science, and Hatchery of Freshwater Aquaculture Research Centre (FARC), Kuku Camp, Sudan. In the Eastern Region of the Nile, which lies between (Latitude 32°67' North and Longitude 15°29' East) in Khartoum North, Khartoum State, Sudan. In an area of 140 m^2. During the experimental work, the temperature ranges from 22 during winter to 45°C during summer, the relative humidity is 5.9% and the rainfall is 10-145 mm/year.

Experimental design and conditions

All the experimental procedures including the animal experimentation were approved by the institutional ethical committee of the Department of Fisheries and Wildlife Science, Sudan University of Science and Technology, Khartoum, Sudan (SUST-SRC-ES-20180807/1). Three hundred Nile tilapia (*O. niloticus*) fingerlings (1.74±0.02 g) were transferred to a laboratory and acclimated to the basal diet for 14 days. Fishes were distributed in five experimental treatments in concrete ponds at a density of 60 fish per group. Aeration was provided by an air pump for each pond. Water was changed partially every 3 days and entirely every week. Fish was fed at a level of 3% of body weight three times a day (9, 13 and 17 o'clock) for seven weeks.

Experimental diets

Five experimental isocaloric (4382.76 Kcal/kg/gross energy) and isonitrogenous (30.2% CP) diets were formulated (Table 1). The control diet (D1) had no probiotic supplement. Diets 2-5 were formulated to be D2 (*B. subtilis* NIOFSD017, 10^7 CFU/g) D3 (*L. plantarum* NIOFSD018, 10^7 CFU/g), D4 (mixture of NIOFSD017, 0.5×10^7 CFU/g and NIOFSD018, 0.5×10^7 CFU/g) and D5 (*S. cerevisiae* NIOFSD019, 10^4 CFU/g) these strains represent three complementary probiotic mechanisms, offereing a broad spectrum of benefits *B. subtilis* (digestive enzyme production and pathogen control) *L. plantarum* (Gut colonization and immune modulation) and for *S. cerevisiae* (Immunostimulant and metabolic enhancer) (Tayyab et al. 2025). The dry ingredients were mixed with corn oil and the microbial isolates were incorporated into the feed diet components as shown in Table 1 (Salinas et al. 2005). After a desirable dough quality was obtained, diets were passed through a mincer with a die (2 mm diameter) and the resulting spaghetti-like strings were dried until the moisture levels were at approximately 10%. The diets were then stored in a -15°C freezer until being used for further studies.

Table 1. Formulation and composition of the experimental diets (dry matter basis)

Ingredient (%)	Experimental diets				
	D1	D2	D3	D4	D5
Fishmeal	40.0	40.0	40.0	40.0	40.0
Sorghum meal	10.0	10.0	10.0	10.0	10.0
Wheat flour	10.0	8.0	8.0	8.0	8.0
Vegetable oil	6.0	6.0	6.0	6.0	6.0
Vitamin mix	3.0	3.0	3.0	3.0	3.0
Mineral mix	1.5	1.5	1.5	1.5	1.5
Wheat bran	10.0	10.0	10.0	10.0	10.0
Cornmeal flour	05	4.0	4.0	4.0	4.0
Groundnut cake	14.5	14.5	14.5	14.5	14.5
Probiotic	-	3.0	3.0	3.0	3.0
Total	100	100	100	100	100

Chemical composition	Experimental diets				
	D1	D2	D3	D4	D5
Dry matter (%)	94.61	95.51	95.60	95.66	95.56
Crude protein (%)	14.30	14.32	14.25	13.95	14.33
Crude fat (%)	7.22	7.09	7.41	7.46	7.42
Ether extract (%)	4.40	4.21	4.53	4.47	4.43
Ash (%)	27.48	27.66	27.58	28.30	28.30
Nitrogen-free extract (%)	42.23	42.45	42.83	42.13	42.13

Note: -: Absent

Table 2. Water quality parameters during the experimental period

Parameters	Average	Apparatus/Methods
Temperature	25.0-28.5°C	Digital thermometer
Dissolved oxygen	6.5-7.4 mg/L	Mobile digital DO-meter (Yellow Springs, OH, USA)
Free carbon dioxide	7-8.4 mg/L	Co2 Test Kid (Chemical Titration Method)
pH	7.2-7.7	Portable pH meter Model Mi 105
Total alkalinity	75- 83.5 mg/L	Chemical method according to (APHA 1992)

Water quality parameters

Water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity during the feeding trials were recorded, based on daily measurements, following standard methods (APHA 1992). The average water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity over the 7-week feeding trial, based on daily measurements in Table 2.

Proximate composition analyses

After a starvation of 24 h, a randomized samples of 25 fishes were killed by dipping in MS-222 solution (200 mg/L), minced and six subsamples of initial body composition were analysed. At the end of the trial, nine fishes from each replicate of dietary treatments were sampled, killed, pooled separately and frozen at -20°C for analyzing the biochemical composition. Six subsamples of the pooled samples of each replicate (n=3×5) were subjected to proximate composition analysis. Proximate composition of the diets, initial and final carcass was worked out using standard protocols (AOAC 2005) as described in a previous investigation (Zehra and Khan 2019). BIOBASE High Automatic Oxygen Bomb Calorimeter (Biobase Meihua Trading Co., Ltd., China) was used for estimating the gross energy content of experimental diets.

Blood collection and analysis

Blood from the caudal vein of 9 fish per replicate was randomly collected and pooled for analyzing hematological parameters. The procured blood was transferred to heparinized Eppendorf tubes. The three subsamples (n=3×3) of pooled samples from heparinized tube were utilized for the assessment of the RBCs and Hb following the methods of Vani et al. (2012). The Hct level was examined by centrifuging fresh blood at 3600 g for 6 min in Micro Hematocrit Centrifuge (SH-120 High Speed Micro Hematocrit, China).

Estimation of liver superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities

Liver was taken off from five starved fish of each replicate tank, pooled and five subsamples (n=3×5) were utilized for assessing the SOD, CAT and GPx activities following the procedures of Takahara et al. (1960), Misra and Fridovich (1972), and Rotruck et al. (1973), respectively. The procedure used by the researcher (Qian et al. 2015) was utilized to evaluate the tissue protein content for the calculation of enzyme-specific activities.

Diet performance evaluation

The effects of replacement of fish meal by probiotics in diets for fingerling *O. niloticus* during the present experiment were assessed by considering the growth and conversion efficiency indices as follows:

Live Weight Gain (LWG%) = Final individual body weight - Initial individual body weight/Initial individual body weight × 100

Specific Growth Rate (SGR%) = ln Final body weight - ln Initial body weight/No. of experimental days × 100

Feed Conversion Ratio (FCR) = Feed fed/Weight gain

Protein Efficiency Ratio (PER) = Weight gain/Protein fed

Protein Deposition of whole fish (PD%) = Final body weight (g) × final body protein (%)-Initial body weight (g) x initial body protein (%)/Protein intake (g) × 100

Survival Rate (SR%) = (Final number of fish/Initial number of fish) ×100

Statistical analysis

The growth data were subjected to one -way analysis of variance (Sokal and Rohlf 1981). Before analysis, data were tested for normality using the Shapiro-Wilk test and the homogeneity of variance using Levene's test for equality of variances. When a significant treatment effect was observed, Tukey's honest significant difference test was used for multiple mean comparisons at a $P<0.05$ level of significance.

RESULTS AND DISCUSSION

The amino acid and fatty acid composition of experimental diets was also affected by the different types of probiotics (Tables 3 and 4). Amino acid composition of the experimental diets was not significantly affected among the varying groups of probiotics. The fatty acid composition of the test diets was also not affected significantly except eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), which were found to decrease in D4 and D5 significantly ($p>0.05$).

Over the seven-week feeding trial. Addition of probiotics to tilapia (*O. niloticus*) fingerlings feeds D4 (mixture of NIOFSD017, 0.5×10^7 CFU/g and NIOFSD018, 0.5×10^7 CFU/g) and D5 (*S. cerevisiae* NIOFSD019, 10^4 CFU/g) were found to be feasible significant differences among the live weight gain (252.87 and 270.11%), feed conversion ratio (1.54), protein efficiency ratio (1.43), specific growth rate (2.25-2.34%) and protein deposition (22.46-25.68%) of fish fed diets D4 and D5 (Table 4). However, in D1 (control diet) and D2, D3 resulted in a significant decrease ($P<0.05$) in growth and conversion efficiencies. Significantly ($P>0.05$) poorest LWG (229.89

and 238.37 and 248.55%), but on the other hand, FCR was found to be the best in D2 (1.64), low PER (1.35, 1.37 and 1.40), SGR (2.13, 2.18 and 2.23%) and PD (21.85, 21.42 and 22.28). Body composition of the fish was significantly altered by the different groups of probiotics (Table 5). No remarkable differences in moisture content were evident in fish fed diets D4 and D5. However, remarkable variations in body moisture were detected in fish fed diets D1, D2 and D3 compared to those of D4 and D5. Ash content differed insignificantly among the groups. No significant differences amongst the body protein of the fish fed diets of D1, D2 and D3. Whereas in fish fed diet, D1 showed a sharp ($P<0.05$) decline in body protein. No remarkable differences ($P>0.05$) in body fat were evident in fish fed diets D1, D2 and D3. However, a significant increase ($P<0.05$) in body fat was noted in fish fed diets D4 and D5. In the current investigation, survival was not significantly affected among the variable treatments.

The effects of different groups of probiotics on liver SOD, CAT, GPx activities and hematological (Hct, RBCs and Hb) tools in fingerling *O. niloticus* are mentioned in Table 6. The almost constancy ($P<0.05$) of liver SOD, CAT and GPx activities in D4 and D5 was recorded and, thereafter, a declining trend was noted in fish fed diets D1, D2 and D3. The stable response of Hct, RBCs, and Hb was noted in fish fed diets D4 and D5. While a declining trend for blood tools was recorded in D1, D2 and D3 in Table 7.

Discussion

Probiotics have gained increasing attention in aquaculture as eco-friendly feed additives that can enhance growth performance, health, immune function, and water quality in *O. niloticus* culture systems. Recent studies consistently show positive effects of probiotic supplementation on tilapia performance metrics and physiological responses (Asha et al. 2024; Paritova et al. 2024; Diab et al. 2025).

Table 3. Amino acid composition (g kg^{-1} dry matter) of the experimental diets[1,2]

	Experimental diets				
	D1	**D2**	**D3**	**D4**	**D5**
Arginine	25.1±0.50	25.2±0.1	25.3±0.1	25.1±0.02	25.3±0.01
Histidine	7.9±0.1	7.9±0.4	7.8±0.2	7.9±0.2	7.8±0.3
Isoleucine	12.1±0.2	12.2±0.4	12.3±0.1	12.2±0.4	12.4±0.4
Leucine	22.3±0.5	22.2±0.4	22.6±0.2	22.3±0.1	22.2±0.1
Lysine	21.3±0.2	21.1±0.2	21.3±0.2	21.1±0.4	21.2±0.2
Methionine	5.7±0.2	5.9±0.1	5.8±0.4	5.6±0.2	5.8±0.2
Cystine	2.5±0.1	2.6±0.1	2.6±0.1	2.0±0.1	2.4±0.2
Phenylalanine	14.6±0.1	14.3±0.1	14.2±0.3	14.5±0.3	14.3±0.3
Tyrosine	13.0±0.2	13.0±0.4	13.1±0.1	13.1±0.1	13.2±0.3
Threonine	14.3±0.1	14.3±0.5	14.2±0.3	14.1±0.2	14.5±0.0
Tryptophan	2.5±0.1	2.4±0.3	2.3±0.1	2.2±0.1	2.1±0.1
Valine	15.5±0.5	15.5±0.2	15.5±0.2	15.5±0.1	15.2±0.2

Note: [1]: Mean values of three replicates±SEM, [2]: Not statistically significant ($P>0.05$)

Table 4. Fatty acids profile of the experimental diets (g kg^{-1})[1,2]

Fatty acid		Experimental diets				
		D1	**D2**	**D3**	**D4**	**D5**
Sat						
Myristic	14:0	8.3±0.1	8.0±0.2	8.5±0.1	8.2±0.3	8.1±0.1
Palmitic acid	16:0	68.7±1.0	67.1±3.0	69.8±7.0	70.4±3.0	72.4±4.0
Stearic acid	18:0	23.0±1.0	22.2±5.0	23.0±1.0	22.9±5.0	22.3±2.0
Mon						
Palmitoleic acid	16:1 n-7	14.2±1.0	14.4±2.0	14.6±2.0	13.9±1.0	13.9±3.0
Oleic acid	18:1 n-9	143.1±4.0	144.2±8.0	149.1±4.0	151.3±1.0	152.1±1.0
Gadoleic acid	20:1 n-11	13.8±1.0	13.6±1..0	13.5±2.0	12.8±3.0	12.6±1.0
Erucic acid	22:1 n-9	11.1±0.2	11.5±0.1	10.9±0.4	9.8±0.8	9.6±0.9
n-3 LC-PUFA						
Linoleic acid (LA)	18:2 n-6	182.1±3.0	182.6±6.0	183.3±1.0	182.8±3.0	182.8±3.0
Gamma-Linolenic acid (GLA)	18:3 n-6	0.2±0.1	0.2±0.02	0.2±0.1	0.2±0.0	0.2±0.0
Arachidonic acid	20:4 n-6	1.6±0.2	1.6±0.1	1.6±0.1	1.6±0.2	1.6±0.1
Alpha-Linolenic acid (ALA)	18:3 n-3	22.5±1.0^{e}	23.7±2.0^{d}	25.8±1.0^{c}	27.5±4.0^{b}	28.7±4.0^{a}
Stearidonic acid	18:4 n-3	3.1±0.1	3.0±0.1	2.7±0.3	2.5±0.1	2.5±0.2
Eicosapentaenoic acid (EPA)	20:5 n-3	15.4±2.0^{a}	15.2±2.0^{a}	14.7±2.0^{ab}	13.5±4.0^{ab}	13.5±4.0^{ab}
Docosapentaenoic acid (DPA)	22:5 n-3	5.3±0.1^{a}	5.1±0.1^{a}	4.6±0.4^{b}	4.4±0.2^{c}	4.4±0.2^{c}
Docosahexaenoic acid (DHA)	22:6 n-3	20.0±3.0^{a}	20.9±1.0^{a}	19.8±2.0^{a}	18.2±2.0^{b}	17.3±3.0^{c}

Note: [1]: Mean values of three replicates±SEM, [2]: Not statistically significant ($P>0.05$)

Table 5. Growth performance and per cent survival of fingerling *O. niloticus* fed diets containing probiotics[1,2]

	Experimental diets				
	D1	**D2**	**D3**	**D4**	**D5**
Initial weight (g fish^{-1})	1.74±0.03	1.72±0.02	1.73±0.01	1.74±0.01	1.74±0.01
Final weight (g fish^{-1})	5.74±0.4^{d}	5.82±0.1^{c}	6.03±0.1^{b}	6.14±0.3^{a}	6.44±0.1^{a}
Absolute weight gain (g fish^{-1})	4.0±0.2	4.1±0.1	4.3±0.1	4.4±0.2	4.7±0.2
Live weight gain (%)	229.89±1.4^{e}	238.37±1.2^{d}	248.55±1.4^{c}	252.87±1.6^{b}	270.11±2.4^{a}
Feed conversion ratio	1.87±0.03^{a}	1.64±0.02^{b}	1.52±0.02^{d}	1.54±0.02^{c}	1.54±0.02^{c}
Specific growth rate (% day^{-1})	2.13±0.03^{e}	2.18±0.01^{d}	2.23±0.04^{c}	2.25±0.01^{b}	2.34±0.02^{a}
Protein efficiency ratio	1.35±0.02^{b}	1.37±0.02^{b}	1.40±0.02^{a}	1.43±0.01^{c}	1.43±0.01^{c}
Protein deposition (%)	21.85±0.1^{a}	21.42±0.2^{b}	22.28±0.1^{a}	22.46±0.3^{b}	25.68±0.3^{c}
Protein gain (g/fish)	3.75±0.01^{a}	3.52±0.02^{b}	3.76±0.01^{a}	3.42±0.03^{c}	3.42±0.03^{c}
Protein retention efficiency (%)	8.17±0.5^{a}	8.05±0.3^{a}	8.42±0.2^{a}	8.13±0.1^{a}	8.13±0.1^{a}
Survival (%)	-	95±4.4	100	100	98±2.0

Note: [1]: Mean values of 3 replicates±SEM, [2]: Not statistically significant (P>0.05)

Table 6. Carcass composition (g kg^{-1} wet weight) of fingerling *O. niloticus* fed diets containing probiotics[1,2]

		Experimental diets				
	Initial	**D1**	**D2**	**D3**	**D4**	**D5**
Moisture	742.0±12.0	778.4±5.2^{a}	761.2±7.1^{b}	755.1±8.2^{c}	752.1±6.5^{d}	750.1±6.3^{e}
Crude protein	106.0±2.0	136.1±0.4^{b}	137.2±0.3^{b}	137.8±0.4^{b}	144.7±0.9^{a}	145.7±0.9^{a}
Crude fat	42.0± 1.0	32.6±3.7^{c}	33.4±0.3^{b}	33.2±0.4^{b}	34.1±0.5^{a}	34.1±0.6^{a}
Ash	22.1± 0.3	20.5±1.6	20.1±0.4	20.6±0.1	20.8±0.5	20.7±04

Note: [1]: Mean values of 3 replicates±SEM, [2]: Not statistically significant (P>0.05)

Table 7. Hematological indices and antioxidant status of fingerling *O. niloticus* fed diets containing probiotics[1,2]

	Experimental diets				
	D1	**D2**	**D3**	**D4**	**D5**
Hematocrit (%)	28.22±0.3^{d}	29.9±0.7^{b}	28.9±0.4^{c}	30.24±0.4^{a}	30.23±0.4^{a}
Red blood Corpuscles (10^{6}/mm^{3})	18.7±0.1^{c}	18.1±0.2^{c}	19.8±0.1^{b}	20.9±0.2^{a}	20.8±0.3^{a}
Hemoglobin (g/dl)	90.1±0.1^{b}	90.7±0.8^{b}	90.1±0.4^{b}	96.2±0.6^{a}	96.2±0.6^{a}
Superoxide dismutase (U/mg protein)	711.0±12.0^{d}	754.0±11.0^{c}	793.0±15.0^{b}	822.3±24.0^{a}	823.0±22.0^{a}
Catalase (U/mg protein)	310.0±5.0^{e}	312.0±4.0^{d}	320.0±2.0^{c}	335.0±8.0^{b}	340.0±7.0^{a}
Glutathione peroxidase activity (U/mg protein)	1705.0±23.0^{e}	1712.0±22.0^{d}	1744.0±20.0^{c}	1825.1±1.1^{b}	1863.1±2.4^{a}

Note: [1]: Mean values of 3 replicates±SEM, [2]: Not statistically significant (P>0.05)

Tilapias are very important in world fisheries, and are the second most important group of food fishes in the world. Nile tilapia *O. niloticus* although, although native to Africa, tilapias are cultured in Asia and the Far East, and occupy two rather separate market niches, being a poor man's food fish in different countries (Maclean et al. 2002; Bhujel 2014). Nile tilapia (*O. niloticus*) is a tropical climate fish of considerable rusticity for cultivation, with a delicate flavor, and a good quality of nutritional aspects with low fat content and free of Y-shaped bones (Medri et al. 2009; Ding et al. 2015). For these characteristics this is one of the most cultivated species in the world (Yousif et al. 2019).

Dietary probiotics have been shown to improve growth performance in Nile tilapia through enhanced feed utilization and nutrient absorption. In one recent investigation, supplementation of feed and/or water with probiotics significantly improved growth performance parameters (e.g., weight gain, feed conversion ratio) and digestive enzyme activities compared with fish receiving a control diet without probiotics. The greatest improvements were observed when probiotics were administered both in feed and water, indicating that multiple routes of probiotic delivery can yield synergistic benefits (e.g., growth, survival) in tilapia aquaculture systems. Similarly, a study evaluating commercial probiotics in a biofloc system reported that supplementation increased final weight and weight gain, likely through enhanced intestinal health and nutrient assimilation, although some parameters, such as specific growth rate, did not differ significantly across treatments.

In this study, fish fed (D5) exhibited the highest (P<0.05) values for live weight gain and specific growth rate (% per day). FCR and SGR were better (P<0.05) in fish-fed diets 1 (D1) and (D2). Fish-fed diet D1 exhibited lower (P<0.05) fat and ash contents in the carcass. The protein efficiency ratio, absolute body weight, live weight gains and feed conversion ratio content significantly decreased in fish fed diet D1. Probiotics are defined as live microorganisms introduced into the Gastrointestinal Tract (GIT) through food or water to promote good health by

enhancing the internal microbial balance (Azad et al. 2019). Their utilization in aquaculture has been recognized as an ecologically sustainable method for preventing disease outbreaks, enhancing growth, and improving digestion (Guo et al. 2016; Adel et al. 2017). Probiotics are a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring an improved use of the feed or enhancing its nutritional value, by increasing the host response towards disease, or by improving the quality of its environment (Verschuere et al. 2000; Dog˘an and Ertan 2017). Nowadays, probiotics are also becoming an internal part of aquaculture practices to obtain high production. Although considerably low information is available on probiotics application for fish, they offer benefits to improving immune status and fish production (Cerezuela et al. 2011; Tayyab et al. 2025). Probiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific health-promoting bacteria, which can improve the host's health (Gibson et al. 2003). Based on the studies of Mahious and Ollevier (2005), foodstuffs that reach the colon (e. g., non-digestible carbohydrates, some peptides and proteins, as well as certain lipids) is a candidate probiotic (Yousefian and Amiri 2009). However, most of the studies have focused on non-digestible carbohydrates, mainly oligosaccharides. Synbiotics are nutritional supplements that combine probiotics and prebiotics, enhancing their beneficial effects (Cerezuela et al. 2011).

Probiotics also influence the intestinal morphology and microbiome of tilapia. Dietary supplementation with specific probiotic strains such as *Leuconostoc mesenteroides* and *Lactococcus lactis* significantly improved gut health markers, including lactic acid bacteria counts, intestinal mucus secretion, and antioxidant enzyme activities. These changes correlated with improved growth outcomes and enhanced immune parameters such as serum lysozyme activity, suggesting that probiotics not only bolster nutrient digestion but also modulate host defenses. Moreover, multi-species *Bacillus* probiotics have been demonstrated to alter the intestinal microbiome composition and promote colonization of beneficial bacteria, which may support digestion and immune competence in tilapia.

The use of probiotics and probiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming an integral part of aquaculture practices for improving growth and disease resistance (Rombout et al. 2010). This strategy offers innumerable advantages to overcome the limitations and side effects of antibiotics and other drugs and also leads to high production (Sahu et al. 2008).

In Nile tilapia farming, probiotics have demonstrated several positive effects, including improved immune responses, enhanced growth performance, increased resistance to ammonia, better digestive health, and reduced stress levels (Islam and Rohani 2021). Probiotics strengthen the immune system of Nile tilapia, enabling them to resist diseases and infections more effectively, which results in higher survival rates and overall health improvements (Shija et al. 2023). Additionally, they promote the proliferation of beneficial gut microbes, which support overall health and development of the fish (Zabidi et al. 2021). Probiotics have also been shown to increase Nile tilapia's resistance to ammonia, helping them tolerate the stressors of aquaculture environments more effectively (Cavalcante et al. 2020).

The immunomodulatory effects of probiotic supplementation are also well documented. A recent study found that feeding Nile tilapia with probiotic-enriched diets improved both growth and certain serum biochemical markers and led to better behavioral activity compared to controls. Enhanced immune responsiveness and improved water quality were noted in fish receiving *Bacillus amyloliquefaciens* at an optimal dietary level, showcasing probiotic potential beyond growth performance alone (Ahmed et al. 2024; Raslan et al. 2025). Additionally, in trials where fish were challenged with *Aeromonas veronii*, combining probiotics with essential oils enhanced resistance to infection and reduced oxidative stress, indicating that probiotic supplementation can strengthen disease resistance in intensive culture environments.

In recent years, there has been a growing interest in understanding the mechanism of action of probiotics and probiotics, especially in humans and other mammals. Probiotics have been shown to influence the activity of digestive enzymes, including carbohydrase's, lipases, and proteases (Eshaghzadeh et al. 2015), which contribute to efficient nutrient breakdown. Taoka et al. (2007) reported that the administration of probiotics such as *B. subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and *S. cerevisiae* increased enzyme activity in tilapia. Similarly, Tan et al. (2019) observed enhanced digestive enzyme activity in Nile tilapia when supplemented with *Rummeliibacillus stabekisii* at concentrations of 10^6 and 10^7 CFU·g^{-1}.

Probiotics activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration and route and frequency of delivery. Some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses and, activity participating in the fight against infections; whereas, others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules or modulate the inflammatory/immune response (Cerezuela et al. 2011).

On the other hand, the potential mechanism of probiotics includes a selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production, an increase in the intestinal short chain fatty acids production, an enhanced binding of these fatty acids to G-coupled protein receptors on leucocytes, an interaction with carbohydrates receptors on intestinal epithelial and immune cells, and partial absorption resulting in a local and systemic contact with the immune system (Seifert and Watzl 2007).

These microbiotas play a vital role in regulating mucosal development and tolerance, contributing significantly to the overall health of the fish (Akhter et al. 2015). By reducing the presence of pathogenic organisms in the gastrointestinal tract, probiotics promote a healthy intestinal environment. This allows for the development of a robust intestinal epithelial layer, characterized by minimal mucosal damage and enhanced nutrient absorption. Such improvements in gut health support optimal growth and development, ensuring the overall well-being of tilapia (Merrifield et al. 2010).

Probiotics are known to produce a wide range of inhibitory compounds that exhibit antimicrobial and antiviral properties. These compounds, produced by beneficial gut bacteria in Nile tilapia, include siderophores, bacteriocins, hydrogen peroxide, lysozymes, proteases, volatile fatty acids (such as lactic, propionic, acetic, and butyric acid), organic acids, and extracellular enzymes. These substances collectively play a crucial role in suppressing viral replication (Chauhan and Singh 2019).

The alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immune responses. However, other effects related have been observed, as improve growth performance, feed utilization, digestive enzyme activity, antioxidant enzyme activity, gene expression, disease resistance, larval survival and gut morphology alter the gut microbiota, mediate stress response, improve nutrition, reduce risk of certain cancers (colon, bladder), produce lactase, alleviate symptoms of lactose intolerance and malabsorption (Rombout et al. 2010).

A synbiotic is defined as a combination of probiotic and prebiotic. It is presumed to impart the beneficial effects of both ingredients. Few data are available regarding the application of synbiotics in aquaculture (Zhang et al. 2010). Synbiotics can help to improve health status, disease resistance, growth performance, feed utilization, carcass composition, gastric morphology, and digestive enzyme activities. As such, many commercial dietary formulations now routinely include probiotics or probiotics.

The nutritional benefits of fish and fish oil consumption on human health, including the prevention of cancer, diabetes and heart diseases, have been well established (Jae-Sung et al. 2010). As public awareness about the health benefits of fish consumption continues to increase, the global demand for aquatic foods is also expected to continue to rise (Shamshak and Anderson 2009). Furthermore, the world's population is expected to grow by more than 30% by 2050, resulting in an estimated 2.3 billion more mouths to feed, with the major growth expected in the developing countries where fish is the primary source of protein (FAO 2014). Aquaculture is recognized as the only way to meet these increasing demands for aquatic foods (Bhujel 2014). Half of the seafood consumed worldwide is from commercial fishing (i.e. fish caught in the wild open waters) (FAO 2009). The other half is farm-raised fish grown under controlled conditions known as aquaculture (Bhujel 2014). The amount of fish produced globally from aquaculture rose from 6% in the 1970s to about 50% of the total fish consumed in the world in 2006. Furthermore, aquaculture is also an important income-generating sector of many economies with considerable prospects for job creation, poverty alleviation, community development, and food security. It provides fish for domestic markets and for international markets. The domestic market improves national food security, and production for international markets creates employment, provides income, and brings in foreign exchange, thereby indirectly contributing to national food security. Probiotic application can also contribute to water quality management in tilapia culture. Studies demonstrate that the inclusion of probiotics in both feed and rearing water can lower levels of toxic nitrogenous compounds such as Total Ammonia Nitrogen (TAN) and ammonia (NH_3), thereby creating a more favorable rearing environment for fish growth and survival (Diab et al. 2025).

Several recent studies indicate that dietary probiotics positively influence growth metrics in Nile tilapia. For example, Ahmed et al. (2024) found that tilapia fingerlings receiving probiotics in both feed and water showed significantly enhanced final body weights, weight gain, and specific growth rates compared with control fish, although survival rates were not significantly different across treatments. This suggests that probiotics can optimize nutrient assimilation and growth even under variable water-exchange regimes. The exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems. A major contributor to this intensive production system is the use of manufactured feeds formulated to meet the nutritional requirements of the targeted fish species (FAO 2009). Feeds account for up to 70% of the variable cost of a commercial aquaculture operation (Lim and Webster 2006). The findings of this study indicate that supplementing Nile tilapia diets with *B. subtilis*, *L. plantarum* and *S. cervisiae* has several practical advantages for commercial aquaculture (cost-effectiveness and improved production efficiency, enhancing growth performance). These strains are scalable due to their stability, enhancing fish health and reducing mortality. This study recommends the commerical aquaculture farmers use it due to the previous studies and findings of this research.

In conclusion, the current experiment showed that the addition of probiotics to Nile tilapia (*O. niloticus*) fingerlings feeds produced the best result in terms of growth in D4 (mixture of NIOFSD017, 0.5×10^7 CFU/g and NIOFSD018, 0.5×10^7 CFU/g) and D5 (*S. cerevisiae* NIOFSD019, 104 CFU/g).

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