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*

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Abstract

The performance, efficiency of consumed feed, body chemical composition, survival rate, antioxidant and immunity parameters of Nile tilapia (*Oreochromis niloticus*) reared in probiotic-treated water were studied. Two hundred apparently healthy Nile tilapia (20 ± 0.3 g) juveniles were reared for 70 days in five different treatments, with five replications as the control group (clean water) and four test groups with two probiotics strains (*Bacillus toyonensis* and *Geobacillus stearothermophilus*) at two different levels (1 or 2 × 10⁵ CFU ml⁻¹) applied in rearing water. Fish reared in water supplemented with *G. stearothermophilus* at low level demonstrated significantly enhanced (p < 0.05) growth performances in terms of final body weight (FBW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and relative growth rate (RGR) compared to the control group. In the same context, supplied fish rearing water a lower amount of *G. stearothermophilus* (GS1) remarkably reduced feed conversion ratio values when compared to the control group. In contrary, all other feed efficiency parameters increased significantly when *G. stearothermophilus* (GS1) water was added at low amount in the compartment with the untreated group. Moreover, probiotic water additives significantly reduced the range and median levels of unionized ammonia (NH₃) in water when compared to the untreated group. According to the findings of the body chemical composition, treated tilapia water with a high level of *B. toyonensis* had significantly higher crude protein and fat levels, as well as lower ash levels, than the control group. When compared to the control group, probiotic-water supplementation significantly improved oxidative status and immunological activity at all bacterial dosage levels, with the fish group enriched with a high level of *G. stearothermophilus* recording the maximum values of both antioxidant and immune activity. Finally, results reveal that water treated with *B. toyonensis* or *G. stearothermophilus* as a probiotic promoted Nile tilapia growth and health status, and this technology may be applied to stimulate tilapia productivity in culture farms.

Keywords Probiotic · Tilapia · Performance · Immunity · Antioxidant status

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Introduction

During the past several decades, aquaculture industry has made substantial contributions to the animal protein production sector in almost all country across the globe, accounting for about half of all seafood consumed annually (FAO 2021). The global development of intensive culture farms, with their damaging discharges of organic and inorganic wastes, has resulted in epidemic outbreaks of several infectious diseases, degradation of the natural ecosystem, particularly eutrophication, fishery breakdown, and reduced biodiversity (Ahmed et al. 2019; Shafique et al. 2021). Furthermore, releasing excessive levels of ammonia in association with poor pond management in intensive culture farms may have a severe influence on fish productivity and overall health condition through deteriorating quality of rearing water (Negm et al. 2021; Abdel-Latif et al. 2022). Consequently, stress may depress the immune system and cause oxidative damage of farmed fish (Raza et al. 2022); therefore, developing new techniques to mitigate these harmful effects on fish is essential.

Because of the harmful ecological impact of chemical, synthetic medicine and antibiotics, as well as the creation of mutagenic resistant bacterial strains and deleterious effects on fish health status, their use to suppress infectious diseases or improving water quality is no longer advised (Pepi and Focardi 2021; El-Kady et al. 2022). Thus, the application of eco-friendly water additives, such as probiotic supplements, to enhance the growth, antioxidant status and immune responses of farmed fish species, has gained much more attention during recent years (Zokaeifar et al. 2014; Naiel et al. 2022b). The use of probiotics is one of the alternative strategies to immunological prophylactic management in aquaculture, and it is regarded as a supplemental technique or alternative to the use of vaccinations and drugs (Naiel et al. 2021). Some Bacillus bacteria species such as Bacillus subtilis, B. cereus, B. coagulans, B. clausii, B. megaterium, B. licheniformis, B. circulans, B. aerius and B. polymyxa are the common probiotics employed for growth and health improvement in fish (Jahangiri and Esteban 2018). The application of Bacillus has been revealed to have the most effectiveness influences against infectious diseases, sustain survival, and promote growth for a wide variety of fish species (Sun et al. 2010; Andani et al. 2012; Wu et al. 2012; Adorian et al. 2019; Naiel et al. 2022c). In addition, recent research has shown the efficacy of probiotic water supplements to produce inhibitory chemicals, boost immunity, and prevent pathogen invasion in the ecosystem and fish gut (Cha et al. 2013; Reda and Selim 2015; Sutthi et al. 2018; Zaineldin et al. 2018). Also, Zhou et al. (2010) investigated that supplemented tilapia rearing water with probiotic could enhance growth and enhances immune responses. Specifically, Bacillus toyonensis is a member of the Bacillus cereus group and has been applied for many years as a feed additive of both fish and animals (Markowiak and Śliżewska 2018). Furthermore, B. toyonensis has previously been identified as an antibacterial agent capable of preventing diarrhea infections caused by E. coli strains as well as other infectious diseases such as salmonellosis, campylobacteriosis, and coccidiosis (Yilmaz et al. 2022). Despite the scarcity of knowledge on G. stearothermophilus' function in fish production, it demonstrates powerful effectiveness in biosorption of heavy metals from industrial waste water (Chatterjee et al. 2010) and enhancing water quality physiochemical features (Hlordzi et al. 2020). Till now, the majority of research examining the properties of probiotics in fish aquaculture have employed dietary supplementation, with little consideration given to the potentially positive benefits of direct probiotic administration in water. Thus, the main purpose of the current study was to evaluate the impact of two isolated probiotic strains (B. toyonensis and G. stearothermophilus) on water quality, growth performance, feed utilization, whole fish body proximate constitution, redox status and immune responses in Nile tilapia fish.

Material and methods

Bacterial strains isolation

O. niloticus fish samples were gathered by randomly from earthen ponds nearby Abbassa village. Internal organs (liver and intestine), gills and skin (body slime) swab samples were collected and grown on MRS and M17 agar medium plates for 72 h at 35 °C. During this study, the isolated probiotic strains (Bacillus toyonensis, and Geobacillus stearothermophilus) were cultivated in the laboratory and routinely evaluated for purity based on their morphological and biochemical properties as ascribed by Garrity et al. (2005). The isolated probiotics (B. toyonensis and G. stearothermophilus) were developed and quantified on specific nutrient agar (MRS and M17 agar media plates) employing spore staining with the spread plate technique (Austin et al. 1995).

Molecular identification of the isolated bacterial strains

The extraction and determination of the genomic DNA quantity

The DNA extraction was applied using the i-genomic BYF DNA extraction Mini Kit. As mentioned before, the DNA genomic was extracted from a pure culture of an isolated...
bacterial strain after it was cultivated on Nutrient broth medium overnight (iNtRON Biotechnology Inc., South Korea). According to Sambrook et al. (1989) procedure, the extracted DNA was determined by estimating the UV-absorbance at wave length of 260 and 280 nm using a spectrophotometer (Shimadzu model UV-240) to unify the DNA quantity and purity.

The PCR partial amplification and sequencing of 16S ribosomal RNA gene

The Maxima Hot Start PCR Master Mix (Thermo K1051) was used to amplify the 16S ribosomal gene, and the nucleotide sequences of the 16S primers utilized were 27F primer-5’-AGAGTTTGATCCTGCGCTCAG-3’ and 1492R primer-5’ TACGGTACCTTGTAGCCAGCTT -3’. To each PCR vial containing 10 µL of 2X PCR Master Mix, 2 µL of each used primer (10 pmol/µl) and 2 µL of the purified DNA sample (40 ng/ µl) were blended. Using sterile distilled water, the total volume of the amplification was completed to 20 µl. The amplification protocol was carried out as follows: (1) denaturation at 95 °C for five min; (2) thirty-five cycles each consists of the following segments; (3) denaturation at 95 °C for one min, then (4) primer annealing for two min. at 52 °C and polymerization at 72 °C for two min. Finally, hold the PCR at 4 °C. The amplified DNA products were electrophoresed for roughly 2 h at a constant 100 V on a 1.0 percent agarose gel with 1X TBE (Tris–borate-EDTA) buffer. The detected bands were estimated using a 100-bp H3 RTU ladder (Cat. No.DM003-R500 Genedirex, Taiwan).

DNA purification after PCR amplification of 16S gene and identification of isolated probiotic

MEGAquick-spinTM Plus Total Fragment DNA Purification Kit, iNtRON Biotechnology Inc., South Korea, purified the bands obtained after PCR amplification that were responsible for 16S RNA genes. After DNA separation from the bacterial strains (2isolates) and concentration determination by spectrophotometer, the 16S primers were employed to amplify the region of the rDNA gene. After amplification, approximately 1500 bp was obtained, as illustrated in Fig. 1. Then, the purified PCR products were sequenced using a forward primer on an ABI 3730xl DNA sequencer (GATC Company, Germany) using forward primer; Sequence 1 [Bacillus toyonensis, 16S ribosomal RNA gene, partial sequence, GenBank accession number MZ427468.1] and Sequence 2 [Geobacillus steaothermophilus, 16S ribosomal RNA gene, partial sequence, GenBank accession number MZ427469.1] as indicated in Table 1. All strains were stored at − 20 °C in dehydrated cultured media (LB: Broth, Miller, Luria–Bertani, Sigma-Aldrich, Millipore, SAFC, Milli-Q, Supelco, BioReliance, Roche) containing 15% glycerol (v/v) until use, as reported by Zokaeifar et al. (2014). The density of prepared cell suspensions was estimated using a spectrophotometer at 600 nm and also correlated to colony-forming units (CFU) using a spread plate technique.

Phylogenetic analysis

The DNA sequences of the bacterial isolates were compared to sequences in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST). The sequence was compared to those of reference taxa found in public databases, and the evolutionary distance was calculated using NCBI Neighbor Joining. The phylogenetic trees of the both isolated probiotic strains are listed in Figs. 2 and 3.

Experimental design and rearing conditions

Healthy juvenile O. niloticus were provided by the Abbassa private hatchery, Egypt, and the experiment was conducted at the wet laboratory of fish nutrition and feed technology department, central Lab. for Aquaculture Research, Abbassa, Abu-Hammad, Egypt. The fish were acclimatized for approximately 2 weeks in 1000-l tanks before the start of the trial. After the acclimation period, the average weight of the fish was 20 ± 0.3 g, and the fish were randomly allocated into twenty-five 100-l aquariums, each containing 10 fish. All tilapia fish were fed a commercial diet (32% protein; North Africa ALLER TIL-PRO 32% EX) three times daily (8:00 a.m., 16:00 p.m., and 24:00 p.m.) at 3% body weight. Five treatments were achieved, each with five replications. Each bacterial strains were cultivated in freshwater nutrient broth for 48 h at 37 °C. Fresh colonies were
gathered via centrifugation at 5000 rpm for 15 min, then the obtained supernatants were discarded, and pellets were washed twice in neutral phosphate buffer saline (pH 7.4) before being suspended in PBS at a density of 1 × 10^5 CFU/ml for application. The probiotic strains (*B. toyonensis* and *G. stearothermophilus*) were added to the rearing water in equal portions to achieve a final concentration of around 1 or 2 × 10^5 CFU ml^-1. The lower concentration was chosen based on prior reports (Nikoskelainen et al. 2001; Bernard et al. 2013); the higher level was chosen considering higher doses are frequently assumed to be more beneficial (Zhou et al. 2010). The fish group that did not receive any probiotic supplements served as the control group. The trial was performed for a 70-day duration, and probiotics were supplied to the rearing water twice a week to keep the level in the fish rearing water stable.

### Water quality measurements

Daily, a Pro Quatro Multiparameter Meter (YSI Incorporated, 1700/1725 Brannum Lane, Yellow Springs, OH 45387 USA) was being applied to monitor and recorded dissolved oxygen (DO), temperature, pH, total dissolved solids (TDS), and conductivity (EC) in all aquariums. Also, the total ammonia (TAN) levels were estimated in all rearing aquarium using a specific test kit (Model HI28049, Hach, USA). The unionized ammonia level (NH3-N) was calculated according to the Henderson–Hasselbalch relationship equation when the water pH and temperature were measured (USEPA 2013). Each water sample was recorded at various intervals during the day (08:00, 10:00, 12:00, 14:00, 16:00, and 18:00) on a regular basis.

### Growth parameters and survival percentage

The successful live fish biomass was weighing every 2 weeks to determine the growth indices. The following formulae were applied to calculate the final body weight (FW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), relative growth rate (RGR), and survival rate (SR);

\[
\text{WG} = W_f - W_0,
\]

\[
\text{SGR} = \frac{(L_n W_f - L_n W_0)}{n} \times 100,
\]

\[
\text{DWG} = \frac{W_G}{n},
\]

\[
\text{RGR} = \left( \frac{W_f - W_0}{W_f} \right) \times 100.
\]

\[
\text{SR} = 100 \times \frac{\text{final live number of fish}}{\text{initial live number of fish}}.
\]

### Feed efficiency indices

Total Feed intake (TFI)\( \left( \frac{\text{g feed}}{\text{fish}} \right) = \frac{FI}{N} \),

where FI is the consumed diets during the feeding trial and N is the total fish number.

Feed conversion ratio (FCR)\( = \frac{\text{TFI}}{\text{WG}} \),

Feed Efficiency ratio (FER)\( = \frac{\text{WG}}{\text{TFI}} \),

Protein efficiency ratio (PER)\( = \frac{\text{WG}}{\text{PI}} \),

where PI is the protein intake (g),

Protein productive value (PPV\%)\( = 100 \times \frac{\text{protein gain}}{\text{consumed protein}}. \)

### Whole body chemical analysis

Fish samples were collected at the end of the trial period to determine the proximate analyses of the fish body, including moisture, dry matter, crude protein, ether extract, and ash contents. All chemical components of fish bodies were determined based on a dry matter basis using the AOAC (2005) technique.

### Blood sampling procedure

By the end of the experiment, nine fish from each treated group (three fish per aquaria) were randomly sampled after being fasted for 24 h. Prior to collecting blood samples from the caudal vein using a 1-ml sterile syringe, the fish was sedated for 3 min with 95 mg l^-1 clove oil (Oleum, Cairo, Egypt), as reported by Adeshina et al. (2016). The pooled blood sample was mixed with dipotassium salt of EDTA as an anticoagulant (0.5 mg ml^-1 blood) and transferred

| Strain                  | Accession number | Closest phylogenetic relative       | Identity % |
|-------------------------|------------------|-------------------------------------|------------|
| *Bacillus toyonensis*   | MZ427468.1       | *Bacillus toyonensis*               | 99.28      |
| *Geobacillus stearothermophilus* | MZ427469.1       | *Geobacillus stearothermophilus*    | 99.11      |
into Eppendorf tubes before being centrifuged (3000×g for 15 min) to determine antioxidant and immune markers. The obtained serum was stored at 20 °C until it was needed.

**Antioxidant and immune parameters**

Superoxide dismutase (SOD) and catalase (CAT) concentration in prepared serum were estimated via commercial colorimetric kits (Bio-diagnostic Co., Cairo, Egypt) and following the method reported by Nishikimi et al. (1972) and Aebi (1984), respectively.

Nitric oxide (NO) level was detected as the method described by Rajaraman et al. (1998). The respiratory burst test was determined as ascribed by Siwicki et al. (1985) 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 microtiter 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 (Schäperclaus 1992). 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 microliters 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.

**Statistical procedure**

The Shapiro–Wilk test was applied to prove that the collected and calculated data followed the normal distribution (Razali and Wah 2011). After that, all data were analyzed using one-way ANOVA, and differences between treatments were assessed using Tukey’s range test. All analytical methods were achieved using SPSS v.22, and the obtained results were shown as mean ± standard error.
from the antioxidant and immune activity were subjected to the one-way ANOVA followed by Dunnett’s multiple comparison of group means to compare the probiotic supplemented groups and the control group, so long as the ANOVA showed significance differences.

Results

Growth performance and survival

The effects of two probiotic strains supplemented into tilapia fish rearing water on performance parameters were studied over a 70-day period (Table 2). At the beginning of the trial, there were no detectable significant variations in initial body weight ($P > 0.05$) values between the treatment and control groups. However, at the end of the experiment, there were significant changes ($P < 0.05$ or 0.001) in the final body weight (FW), weight body gain (WG), daily weight gain (DWG), relative growth rate (RGR), and specific growth rate (SGR) of fish between the probiotic treatments and control groups (Table 1). The GS1-treated group had higher values for all growth indicators when compared to the other treated or non-treated groups. In the same context, there was a significant difference ($P > 0.05$) in the survival percentage between the probiotic-treated groups and the control group, whereas both probiotic groups administered with a low bacterial count (BT1 and GS1) exhibited better survival rates.

Feed efficiency

Feed utilization characteristics including feed intake (FI), feed conversion ratio (FCR), feed efficiency ratio (FER), protein intake (PI), protein efficiency ratio (PER), and protein productive value (PPV) of tilapia fish were assessed and statistically examined (Table 3). Significant variations in feed consumption and protein intake were detected in fish reared in water supplemented with higher levels of $B. toyonensis$ (BT2) compared to the untreated group. The fish grown in water supplied with a lower amount of $G. stearothermophilus$ (GS1) had the lowest FCR values when compared to the control group. In contrast, tilapia fish kept in water with a lower quantity of $G. stearothermophilus$ (GS1) demonstrated higher FER, PER, and PPV values than the untreated group.

Body chemical composition

There were no significant variations in the dry matter percentage of all experimental groups, whether probiotic supplemented or unsupplemented (Table 4). While crude protein and lipids levels considerably ($P > 0.05$ or 0.001) improved in all treatment groups as compared to the control group, with the BT2 group exhibiting higher values, followed by GS2 and BT1, respectively. In contrast, all probiotic supplemented groups had significantly ($P < 0.001$) reduced ash concentrations than the control group, with BT2 being the lowest observed group.

Table 2 Data of growth performance and survival of tilapia fish reared in treated water with or without probiotics for 70 days

| Parameters | Treatments | $P$ value |
|------------|------------|-----------|
|            | CTR | BT$_1$ | BT$_2$ | GS$_1$ | GS$_2$ |
| IW (g)     | 20.41 ± 0.13 | 20.47 ± 0.12 | 20.26 ± 0.01 | 20.67 ± 0.03 | 20.51 ± 0.16 | 0.446 |
| FW (g)     | 44.19 ± 0.26 | 54.36 ± 0.75 | 53.10 ± 1.31 | 58.97 ± 0.83 | 52.40 ± 1.04 | < 0.001 |
| WG (g)     | 23.78 ± 0.27 | 33.89 ± 0.83 | 32.84 ± 1.29 | 38.30 ± 0.86 | 31.89 ± 0.97 | < 0.001 |
| DWG (g)    | 0.34 ± 0.04  | 0.48 ± 0.12  | 0.47 ± 0.18  | 0.55 ± 0.12  | 0.46 ± 0.39  | < 0.001 |
| SGR (g d$^{-1}$) | 1.10 ± 0.12 | 1.40 ± 0.26 | 1.38 ± 0.35 | 1.50 ± 0.22 | 1.33 ± 0.25 | 0.001 |
| RGR (%)    | 1.17 ± 0.18  | 1.66 ± 0.47  | 1.62 ± 0.64  | 1.85 ± 0.45  | 1.56 ± 0.05  | 0.013 |
| SR (%)     | 86.67 ± 3.33 | 100 ± 0.00  | 96.67 ± 3.33 | 100 ± 0.00  | 96.67 ± 3.33 | 0.021 |

CTR, control fish group reared in clean water; BT$_1$, fish group reared in supplemented water with $B. toyonensis$ at level 1 × 10$^5$ CFU ml$^{-1}$; BT$_2$, fish group reared in supplemented water with $B. toyonensis$ at level 2 × 10$^5$ CFU ml$^{-1}$; GS$_1$, fish group reared in supplemented water with $G. stearothermophilus$ at level 1 × 10$^5$ CFU ml$^{-1}$; GS$_2$, fish group reared in supplemented water with $G. stearothermophilus$ at level 2 × 10$^5$ CFU ml$^{-1}$; IW, initial weight; FW, final weight; WG, weight gain; DWG, daily weight gain; SGR, specific growth rate; RGR, relative growth rate; SR, survival rate

Within each row, means with the different superscript(s) are significantly different ($P < 0.05$, 0.01 or 0.001); Data were presented as mean ± SE
Water quality criteria

During the trial period, no difference was recorded in the range or median values of water temperature (TEMP), dissolved oxygen (DO), total dissolved solids (TDS), electric conductivity (EC), and pH values due to the administration of different probiotic strains to fish rearing water and the control group (Table 5). Probiotic water additions considerably decreased the unionized ammonia (NH3) range and median values in water when compared to control group.

Immune responses

The effects of enriched tilapia rearing water with two different probiotic strains (BT, *B. toyonensis* and GS, *G. stearothermophilus*) at two levels (1 or 2 × 10⁵ CFU ml⁻¹) on some immune parameters including nitric oxide (NO), NBT, and lysozyme concentrations are illustrated in Fig. 4. When compared to the control group, NO levels in all probiotic-treated groups improved significantly (*P* < 0.001) at any administration level, while the GS2 group recorded the highest NO concentration. On the other hand, the NBT values revealed no significant differences between the treated groups and the control group, with the exception of the BT2 group, which significantly (*P* < 0.05) enhanced the NBT level compared to the untreated group, whereas lysozyme activity increased significantly (*P* < 0.01 or 0.001) in all fish groups supplemented with probiotics as water additives when compared to the control group.

Antioxidative remarks

The influences of fortified tilapia rearing water with two different probiotic strains (BT, *B. toyonensis* and GS, *G. stearothermophilus*) at two levels (1 or 2 × 10⁵ CFU ml⁻¹) on some antioxidative parameters including superoxide dismutase (SOD), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) are illustrated in Fig. 5. When compared to the control group, SOD and GPx activities improved significantly (*P* < 0.001 or 0.01) in all probiotic-treated groups at any administration level, whereas TAC values revealed no significant differences between the treated groups and the control group.

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**Table 3** Feed utilization measurements of tilapia fish reared in treated water with or without probiotics for 70 days

| Parameters | Treatments | CTR | BT₁ | BT₂ | GS₁ | GS₂ | *P* value |
|------------|------------|-----|-----|-----|-----|-----|-----------|
| FI (g)     | 42.43 ± 1.24 | 50.03 ± 0.67 | 52.77 ± 0.52 | 50.67 ± 0.58 | 50.87 ± 0.46 | < 0.001 |
| PI (g)     | 12.58 ± 0.37 | 14.83 ± 0.20 | 15.65 ± 0.15 | 15.02 ± 0.17 | 15.08 ± 0.14 | < 0.001 |
| FCR (g/g)  | 1.89 ± 0.03  | 1.48 ± 0.03  | 1.61 ± 0.06  | 1.33 ± 0.04  | 1.60 ± 0.06  | 0.005    |
| FER (g/g)  | 56.08 ± 1.04 | 67.71 ± 1.13 | 62.21 ± 1.23 | 75.64 ± 1.36 | 62.72 ± 1.22 | < 0.001 |
| PER (g/g)  | 1.89 ± 0.03  | 2.28 ± 0.04  | 2.09 ± 0.07  | 2.55 ± 0.08  | 2.12 ± 0.07  | < 0.001 |
| PPV (%)    | 14.14 ± 1.38 | 17.15 ± 0.63 | 14.89 ± 0.43 | 19.31 ± 0.83 | 14.86 ± 0.52 | 0.001    |

CTR, control fish group reared in clean water

BT₁, fish group reared in supplemented water with *B. toyonensis* at level 1 × 10⁵ CFU ml⁻¹

BT₂, fish group reared in supplemented water with *B. toyonensis* at level 2 × 10⁵ CFU ml⁻¹

GS₁, fish group reared in supplemented water with *G. stearothermophilus* at level 1 × 10⁵ CFU ml⁻¹

GS₂, fish group reared in supplemented water with *G. stearothermophilus* at level 2 × 10⁵ CFU ml⁻¹

*Within each row, means with the different superscript(s) are significantly different (*P* < 0.05, 0.01 or 0.001); Data were presented as mean ± SE

**Table 4** Body chemical analysis of tilapia fish reared in treated water with or without probiotics for 70 days

| Parameters | Treatments | CTR | BT₁ | BT₂ | GS₁ | GS₂ | *P* value |
|------------|------------|-----|-----|-----|-----|-----|-----------|
| Dry matter | 24.43 ± 0.73 | 24.73 ± 0.94 | 25.16 ± 0.16 | 24.42 ± 0.02 | 24.36 ± 0.16 | 0.063    |
| Crude protein | 62.40 ± 0.18 | 64.57 ± 0.12 | 65.68 ± 0.13 | 63.66 ± 0.11 | 64.66 ± 0.03 | 0.002    |
| Crude lipids | 17.43 ± 0.73 | 19.55 ± 0.55 | 19.86 ± 0.69 | 19.32 ± 0.58 | 19.73 ± 0.17 | 0.001    |
| Ash | 18.15 ± 0.32 | 13.88 ± 0.11 | 12.46 ± 0.19 | 15.02 ± 0.85 | 13.61 ± 0.36 | < 0.001 |

CTR, control fish group reared in clean water

BT₁, fish group reared in supplemented water with *B. toyonensis* at level 1 × 10⁵ CFU ml⁻¹

BT₂, fish group reared in supplemented water with *B. toyonensis* at level 2 × 10⁵ CFU ml⁻¹

GS₁, fish group reared in supplemented water with *G. stearothermophilus* at level 1 × 10⁵ CFU ml⁻¹

GS₂, fish group reared in supplemented water with *G. stearothermophilus* at level 2 × 10⁵ CFU ml⁻¹

*Within each row, means with the different superscript(s) are significantly different (*P* < 0.05, 0.01 or 0.001); data were presented as mean ± SE
stearothermophilus) at two different concentrations (1 or 2 × 10^5 CFU ml⁻¹) on some oxidative remarks including catalase (CAT) and super oxide dismutase (SOD) levels are presented in Fig. 5. In compartment with the untreated group, CAT concentrations in all probiotic-treated groups increased significantly (P < 0.001) at any administration level, while the GS2 group exhibited the highest values of CAT activity. Probiotic supplementation of tilapia rearing water significantly increased SOD activity, with low level probiotic addition (BT1 followed by GS1) recording the highest SOD values.

### Discussion

The physicochemical characteristics of fish rearing water are critical indications of water quality and the efficacy of a culture system in sustaining fish output (Negm et al. 2021). The findings of our investigation indicated that all water physicochemical feature ranges were confirmed to be within acceptable limits for tilapia production as previously reported by Boyd and Tucker (2012). Dissolved oxygen (DO) is a vital water quality characteristic that maintains all living organisms, including cichlid fish species (Zink et al. 2011). Furthermore, fish and other aquatic species are found to be stressed, sensitive to infectious diseases and grow slowly under low dissolved-oxygen levels (≥4 mg/l) (Boyd 2011). Our results indicate that probiotic administration into fish rearing water has no effect on DO levels. The capability of Bacillus bacterial strains to modify DO is via lowering of the fish's stress level as indicated in antioxidant levels, resulting in reduced oxygen use in all bacterial-treated groups (Hlordzi et al. 2020). Furthermore, the photosynthetic process is controlled by bacterial water administration, which frees CO₂ and bicarbonates, resulting in increased carbonates and DO level and, consequently, modifying water pH, thereby releasing carbonates raised water pH level via hydrolysis process (Sunitha and Padmavathi 2013). This attribution was found to be confirmed by our findings regarding the preservation of pH levels in all bacterial-treated groups in a profitable range without the need of daily water exchange (exchanged complete water every 2 weeks). Furthermore, these findings are consistent with those of Zhou et al. (2010) study on tilapia fish water treated with probiotic.

Unionized ammonia (NH₃) and other nitrogenous waste are key factors in intensive aquaculture systems (Datta 2012), and recognizing their dynamics is a vital for the sustainability of the aquaculture system and preventing fish from sudden death (Naiel et al. 2022b). Our results show that treated tilapia water could reduce ammonia range and median levels in rearing water. These results found to be in line with Khademzade et al. (2020) findings that administered shrimp pond with two bacterial strains (Pediococcus acidilactici and Bacillus cereus) significantly reduced nitrogenous concentration. In addition, John et al. (2020) reported that introducing probiotic strains (B. amyloliquefaciens, B. cereus, and Pseudomonas stutzeri) into Oreochromis mossambicus rearing water significantly reduced ammonia concentration. The Bacillus species had the ability to remove the different types of nitrogen from fish wastewater (Liu et al. 2020) and play a vital role on modulating nitrogen cycle through ammonification, nitrification, and denitrification as well as nitrogen fixation (Rout et al. 2017; Yousuf et al.

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**Table 5 Water quality criteria of tilapia fish reared in treated water with or without probiotics for 70 days**

| Parameters | CTR | BT₁ | BT₂ | GS₁ | GS₂ |
|------------|-----|-----|-----|-----|-----|
| TEMP (°C)  | 26–28 | 27.14 | 27–28 | 27.57 | 26–28 | 27.42 | 26–28 | 27.17 | 26–28 | 27.22 |
| DO (mg/l)  | 6–6.1 | 6.2 | 5.9–6.4 | 6.2 | 5.9–6.4 | 6.21 | 5.9–6.4 | 6.21 | 6–6.3 | 6.42 | 6–6.4 | 6.15 |
| pH         | 7.1–7.4 | 7.2 | 7.1–7.4 | 7.2 | 7–7.3 | 7.12 | 7–7.4 | 7.18 | 7–7.4 | 7.14 |
| TDS (µg/l) | 297–310 | 311.5 | 312–327 | 319.5 | 397–410 | 401.28 | 450–491 | 468.28 | 491–512 | 506.5 |
| NH₃ (mg/l) | 0.002–0.009 | 0.007 | 0.001–0.006 | 0.004 | 0.001–0.005 | 0.003 | 0.002–0.007 | 0.005 | 0.001–0.009 | 0.005 |
| EC (μS/l)  | 0.49–0.49 | 0.477 | 0.49–0.49 | 0.479 | 0.591–0.612 | 0.602 | 0.069–0.732 | 0.668 | 0.707–0.762 | 0.729 |

CTR, control fish group reared in clean water
BT1, fish group reared in supplemented water with B. toyonensis at level 1 × 10^5 CFU ml⁻¹
BT2, fish group reared in supplemented water with B. toyonensis at level 2 × 10^5 CFU ml⁻¹
GS1, fish group reared in supplemented water with G. stearothermophilus at level 1 × 10^5 CFU ml⁻¹
GS2, fish group reared in supplemented water with G. stearothermophilus at level 2 × 10^5 CFU ml⁻¹

Data were presented as R, rate and M, median; TEMP, temperature; DO, dissolved oxygen; TDS, total dissolved solids; NH₃, unionized ammonia; EC, electric conductivity
For instance, applied *B. amyloliquefaciens* DT into fish pond transformed organic form of nitrogen into ammonium (Hui et al. 2019), which *B. cereus* PB8 could remove nitrite from shrimp rearing water (Barman et al. 2018). The presence of heterotrophic nitrifying bacteria could stimulate the Coenzyme Q to obtain electrons from external carbon sources during the oxidation of ammonia to nitrite. Thus, the elimination of ammonia level in fish rearing water might be linked with the absorption of carbonaceous molecule via improving the heterotrophic bacteria development. In the same context, Li et al. (2019) demonstrated that *Bacillus cereus* showed higher carbon dioxide uptake and ammonia removal capacities by activating the heterotrophic nitrification pathway.

In the existing research, the use of two separate probiotic strains in the fish culture water had a positive influence on the growth and efficiency of consumed feed in Nile tilapia. Early reports have also shown that enriched Nile tilapia rearing water with probiotics markedly improved growth and promoted productivity (Kord et al. 2021, 2022; Van Doan et al. 2021; Deng et al. 2022). Furthermore, Kord et al. (2022) indicated that incorporating probiotics into fish water increased the total count of *Bacillus* spp. in fish gastric tract, indicating that probiotics might...
play a significant role in promoting fish growth, health, and immunity. Moreover, Ghanei-Motlagh et al. (2021) confirmed that the relation between inclusion of probiotics into rearing water and improving the nutritional state of fish could be attributed to the ability of *Bacillus* spp. as a probiotic on promoting the digestive enzymes secretions, modulating intestinal microbial population, generated some vitamins, stimulating appetite and ingested the nutrient components that had not been digested. Also, the relevance of *Bacillus* sp. as a probiotic and its function in promoting fish growth, gut health, and immunity is well previously documented (Kuebutornye et al. 2019; Maas et al. 2021).

Superoxide dismutase (SOD) and catalase (CAT) activity are essential oxidative mediators that assist fish in dealing with the harmful effects of intracellular oxidative stress. Biologically, they could help to preserve the cell homeostasis through eliminating any abnormalities induced by free radical production (Naiel et al. 2022a). For instance, SOD catalyzes the superoxide radicles dismutation to alleviate their hazards (Islam et al. 2021), whereas CAT ameliorate fish cells from damage caused by the accumulation of free radicals (David et al. 2008). According to our CAT and SOD activities results, the inclusion of several probiotic strains in the fish raising water considerably prompting the fish's protection capability against oxidative damage. Similar results have been reported that when *Bacillus* spp. supplied as water additives (alone or mixture and live or heat-killed), the redox status was improved largely to some extent in some fish species (Taoka et al. 2006; Ye et al. 2011). Higher levels of both antioxidant enzymes might be attributed to *Bacillus* bacterial strains' ability to reduced algae growth, decreased organic load, increased nutrient concentration, increased beneficial bacterial population, inhibition of potential pathogens, and increased dissolved oxygen concentration (Verschuere et al. 2000; Hlordzi et al. 2020).

Specifically, probiotic-enriched fish rearing water was revealed to be linked to probiotic bacteria's ability to improve water quality (Mohammadi et al. 2021; Tabassum et al. 2021), which indirectly promotes oxidative status and immune response of cultured fish.

Owing to our research results, the inclusion of probiotics into fish rearing water significantly boosted nitric oxide (NO), and LYZ activities. The NO has an active protective role against virus infections, stimulating viral clearance and fast host recovery (Eddy 2005). Furthermore, LYZ is a key component of the innate immune defense mechanism, which might be activated to combat pathogenic bacterial infections (Li et al. 2021). Significant increases in the activity of both immunity indices in the present investigation demonstrated the immunostimulatory action of these probiotic strains in boosting the fish immune system and enhancing the fish's ability to eliminate infections. Also, improvement in LYZ activity indicated that supplied fish rearing water with probiotics may promote the fish's lytic activity against gram-positive and gram-negative disease-causing bacteria (Kord et al. 2022).

In the same context, El-Kady et al. (2022) demonstrated that applied commercial probiotics into fish rearing water significantly stimulate LYZ activity as well as phagocytic activities. In fact, it is well known that the cell wall of some *Bacillus* bacterial strains are covered by a thick layer of a murein (peptidoglycan) (Tocheva et al. 2013), that have the ability to...
produce LYZ (Höltje 1996). Thus, these findings may reflect the various features of the probiotics employed.

Conclusion

The current trial exhibits that fish reared in water supplied with two different probiotic Bacillus spp. strains (B. toyonensis and G. stearothermophilus) had higher growth performance and feed efficiency than the control group, as well as stimulate immunity and antioxidant status and enhanced water quality criteria. However, additional research into the possibility of adding probiotics into water to promote fish health is required.

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Data Availability All related data were available under reasonable request from the corresponding author.

Code availability Not applicable.

Declarations

Ethics approval The animal ethics guidelines were followed and approved from the Zagazig university animal ethics committee.

Consent to participate All the authors equally participated to prepare the manuscript in all stages.

Consent for publication All the authors approved to submit the final version of the manuscript to Applied Water Science Journal.

Conflict of interest The authors declare that they have no competing interests.

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