Nano spirulina dietary supplementation augments growth, antioxidative and immunological reactions, digestion, and protection of Nile tilapia, Oreochromis niloticus, *against* Aeromonas veronii *and some physical stressors*

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Nano spirulina dietary supplementation augments growth, antioxidative and immunological reactions, digestion, and protection of Nile tilapia, *Oreochromis niloticus*, against *Aeromonas veronii* and some physical stressors

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Abstract The current study evaluated the effects of nano delivery of *Spirulina platensis* on growth performance, digestive enzymes, and biochemical, immunological, and antioxidative status, as well as resistance to *Aeromonas veronii* and some physical stressor challenges in Nile tilapia, *Oreochromis niloticus*. Three experimental fish groups (n = 270) with mean weights of 26 ± 0.30 g and mean lengths of 10 ± 0.5 cm were used; the first additive-free basal diet served as the control group, whereas the following two groups were supplemented with spirulina nanoparticles (SPNP) at 0 (control), 0.25, and 0.5%/kg diet for 4 weeks. Following the feeding trial, fish were challenged with hypoxia, cold stresses, and pathogenic bacteria (*A. veronii*) infection (9×10^8 CFU/ml). SPNP supplementation, especially 0.5%, ($p < 0.05$) significantly increased growth performance (specific growth rate % day^{-1}, feed conversion ratio, and length gain rate %), immunological (plasma lysozyme and liver nitrous oxide) antioxidants (superoxide dismutase, catalase, and glutathione peroxidase in liver), biochemical (aspartate aminotransferase, alanine transaminase, glucose, and cortisol concentrations in plasma) assays, and digestive enzymes (lipase and amylase in plasma). The expression of liver's heat shock protein 70 (HSP70) and interleukin 1, beta (IL-1β) genes showed a significant upregulation outline of 0.5% SPNP > 0.25% SPNP > 0% SPNP compared with the control. Protection in the incorporated fish groups exposed to *A. veronii* was 100% compared with the control group, which showed 50% cumulative mortalities. In conclusion, dietary SPNP supplementation improved growth performance, antioxidant activity, immune response, digestive enzymes, related gene expression, and resistance of Nile Tilapia to hypoxia, cold, and *A. veronii* infection. Thus, SPNP could be used as a natural therapy for controlling those stressors.

Keywords Nano *Spirulina platensis* · Immune response · Growth performance · Gene expression · Hypoxia · Cold · *Aeromonas veronii* · *Oreochromis niloticus*

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Introduction

Nile tilapia *O. niloticus* is one of the top aquaculture species in many countries, including Egypt, where it accounts for about 75.54% of the total aquaculture

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production. Egypt is the world's third largest tilapia producer after China and Indonesia (Shaheen 2013; FAO 2016; Fitzsimmons 2016). Nile tilapia *O. niloticus* culture faces great challenges, among which bacterial infection is considered one of the most significant threats to successful production (Aly et al. 2016). *A. veronii* is reported to have caused severe mortalities in cultured Nile tilapia in Egypt and the mortality rate reached 100% within 24 h, for the 8.9×10^6 CFU/ml dose that killed 100% of experimental fish (Dong et al. 2017). Moreover, physically stressful conditions weaken the immune system, make the fish more susceptible for infection, and increase disease incidence. Winter season conditions in Egypt are among the most stressful conditions for tilapia, decreasing survival and rendering hatcheries unable to produce fry to be stocked in the ponds during winter (Aly et al. 2016). Those stressful conditions are often associated with oxidative stress because fish are unable to detoxify the reactive oxygen species (ROS) (Dong et al. 2017).

With the arising risks and limitations associated with antibiotics and chemical therapeutic usage in aquaculture, attention is currently being paid to the dietary combination of nature-friendly alternatives (Song et al. 2014). *Spirulina platensis* is a filamentous cyanobacterium (blue-green algae) that contains several dietary components such as protein, B vitamins (mainly riboflavin), necessary minerals (mainly iron), essential amino acids, polyunsaturated fatty acids (PUFA), chlorophyll, carotenoids, minerals, and a few pigments (i.e., phycocyanin and allophycocyanin) (Palmegiano et al. 2008; Güroy et al. 2012). *S. platensis* is reported to possess enhancing properties and have positive effects on growth performance, on body composition through its rich content of protein and PUFA, and on immunity and disease resistance in different fish species such as the African catfish *Clarias gariepinus* and Nile tilapia *O. niloticus* (Promya and Chitmanat 2011; Ibrahem et al. 2013; Abdelkhalek et al. 2017) through its content of pigments that possess antioxidative properties and are able to scavenge peroxide radicals (Palmegiano et al. 2008).

Nanotechnology is the work on elements at the nanoscale generally ranging between 1 and 100 nm (10^{-9}–10^{-7m}), and scientifically those elements are known as nanoparticles. Nanoparticles are well known to display unique characters and have the ability to stay in the blood stream for long periods, increasing its availability and effectiveness (Nair et al. 2016; Hill and Li 2017).

To the best of our knowledge, no previous studies have reported the effect of spirulina nanoparticles (SPNP) on growth, immune, and antioxidative stress responses in fish. Thus, the current study aimed to investigate the effect of dietary SPNP on growth performance, digestive enzyme activity, and antioxidant and immune responses of Nile tilapia in response to hypoxia, cold, and *A. veronii* infection challenges.

Materials and methods

Diets preparation

S. platensis was obtained in powder form from the Algal Biotechnology Unit, National Research Centre, Giza, Egypt. It was prepared in nano form in the Egyptian Nanotechnology Center (EGNC), Cairo University's new campus, Sheikh Zayed City (a suburb of Cairo, Egypt), using the top-down technique for green synthesis of *S. platensis* nanoparticles with the help of the ball mill method according to the EGNC protocols. Briefly, the series of ball mills varied in size (1 cm, 0.5 cm, and 0.25 cm). The ratio of weight of ball mills to weight of *S. platensis* particles was control constant 10:1, and the volume of ball mills to volume of dill seed particles was control constant 5:1 g. The current ball mills in our experiments consist of a stainless steel vial mounted on a vibrating plate. Stainless steel balls crash repeatedly with the plate and the powder inside the drum for a period of 36 h.

Spirulina nanoparticle (SPNP) concentrations of 0.25 and 0.5% SPNP/kg diet were chosen, following those of the traditional form of *Spirulina* (Mahmoud et al. 2018), and were incorporated in tilapia commercial fine basal diet (*KOUDIJS® Tilapia fish feed 30%*, *Kapo feed*, *Alexandria*, *Egypt*, Table 1). All ingredients were mixed using a mixer (Mienta, France) and the obtained pellets were air dried at room temperature and then stored at 4 °C until use.

Fish

The current study was performed in the Aquatic Animals Diseases and Management Department, Benha University, Egypt. *Oreochromis niloticus* (n = 270, initial body weight = 26 ± 0.30 g, and initial length = 10 ± 0.5 cm) was obtained from a private fish farm at Kafr El Sheikh Governorate, Egypt. Fish were inspected externally according to Klemm et al. (1993) and stocked in a 750-L fiberglass tank, receiving control diet during the acclimatization period of 10 days. Fish were randomly allocated to three experimental groups and

distributed into 750-L tanks (triplicate design, 90 fish/group, and 30 fish/replicate). Fish were fed ad libitum twice daily (at 09:00 a.m. and 17:00 p.m.) for 30 days. Throughout the experiment, water temperature and dissolved oxygen were monitored daily and adjusted at 26.0 ± 0.5 °C and 6.0 ± 0.20 mg/L, respectively. Unionized ammonia (NH_3) level was 0.26–0.33 mg/L and pH was 7 ± 0.3.

Growth performance

Specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and length gain rate (LGR, %) were calculated as follows:

Specific growth rate (SGR) (% day^{-1}) = [(ln final body mass in g) − ln initial body mass in g)/number of trial days] × 100.

Feed conversion ratio (FCR) = $F/(Wf - Wi)$; F is weight of feed offered to fish, Wf is the fish's final weight, and Wi is the fish's weight at stocking.

Length gain rate (%) = 100 × [Average terminal body length (cm) − Average initial body length (cm)]/Average initial body length (cm).

Stressors challenge

Hypoxia and cold challenges

At the end of the experiment (30 days), 15 fish/group (five fish per replicate) were randomly transferred to a 50-L tank system for application of cold and hypoxia stressors as previously addressed by Elabd et al. (2017).

Table 1 Ingredients and composition of the experimental basal diet

Composition (% dry weight)	
Moisture	10.5
Crude protein	30
Crude lipid	5.30
Crude fiber	5.80
Ash	10 Maximum
Mineral premix[a]	2
Vitamin premix[b]	1

[a] Mineral premix (mg/kg diet): Zn, 31.0; Cu, 1.2; Mn, 5.0; Fe, 1.3; Se, 0.14; Co, 0.28

[b] Vitamin premix (IU /kg and mg/kg diet): vitamin D, 8000 IU; vitamin A, 15 000 IU; vitamin K, 11; ascorbic acid (35%, 800; folic acid, 7.0; biotin, 0.55

Briefly, cold challenge was applied by moving fish from 26.0 ± 0.5 °C water temperature to 12 °C pre-cooled aerated water in the 50-L tanks for 30 min, while hypoxia challenge was performed by shutting off the aeration, lowering the water column and monitoring for hypoxia signs (this took several hours and dissolved oxygen was around 1.5–3.0 mg/L).

Bacteria and challenge experiment

At the end of the 30-day feeding trial, five fish per replicate (n = 15 fish/group) were injected intraperitoneally with pathogenic *A. veronii* at a density of 0.2-mL (9×10^8) colony-forming units per ml (CFU/ml) by using McFarland standard tubes following El Latif et al. (2019). That bacterial strain was previously isolated from moribund fish at the Department of Aquatic Animals Diseases and Management, Faculty of Veterinary Medicine, Benha University. That isolate was identified and its pathogenicity was confirmed by the API20E system (Biomerieux, France). Mortality was recorded on a daily basis for 15 days. Clinical signs and postmortem lesions were also recorded. The cumulative mortality percentage was calculated according to Anderson et al. (1980).

Sampling

At the end of the 30-day feeding trial and after hypoxia and cold challenges, blood samples were collected using a 1-mL heparinized syringe (9 fish/group, 3 fish/tank) after being euthanized using 250 ppm in water tricaine methanesulfonate (MS222) (Syndel Laboratories, British Columbia) following the same procedures previously described by Elabd et al. (2016) and centrifuged at 3600 rpm at 4 °C for 5 min to separate plasma for the evaluation of glucose and cortisol concentrations, digestive enzymes (lipase and amylase), and lysozyme activity. Following plasma collection, liver tissues were collected and divided into two parts. The first part was collected in phosphate-buffered saline (PBS), pH 7.4 for assaying nitrous oxide, superoxide dismutase, catalase and glutathione peroxidase, aspartate aminotransferase, and alanine transaminase; the second part was sampled in RNAlater (Ambion, USA) and kept at − 80 °C for gene expression assessment.

Immunological parameters

Nitrous oxide and lysozyme activities were measured spectrophotometrically at 570 and 450 nm, respectively. Lysozyme activity was measured using lysozyme detection kits (Sigma-Aldrich, USA) according to the company protocol and the methods described in our previous studies (Elabd et al. 2016, 2017). Nitrous oxide was measured using oxidation of nitrite that correlates with the amount of NO, using 85-μl sample mixed with 85-μl Griess reagent (Sigma-Aldrich, USA) following Reda et al. (2018).

Antioxidants and biochemical measurements

SOD, CAT, and GPx activities were assayed according to procedures previously followed by Aebi (1984) using a 50-μl sample volume. SOD measurement depended on the ability of the enzyme to inhibit the phenazine methosulphate with nitro blue tetrazolium dye as an indicator at 340 nm. CAT also included the measurement of a reduction of hydrogen peroxide concentration at 540 nm. GPx was estimated through calculating the enzyme amount that will oxidize 1.0 nmol of NADPH to NAD^{P+} per minute at 25 °C and 340 nm.

Glucose and cortisol concentrations, as well as AST and ALT activities, were measured using 20 μl spectrophotometrically at 340 nm following the decrease in absorbance kinetically within 5 min according to Huang et al. (2006) and Liu et al. (2014).

Digestive enzymes

Activities of digestive enzymes (lipase and amylase) in plasma were measured following the company's protocol (Cusabio Biotech Co. Ltd., China) using the diagnostic reagent kits.

Gene expression

Gene expression analysis was performed according to the method described in our previous studies (Elabd et al. 2016). Trizol method was used to isolate total RNA from liver samples using 1-ml Trizol (Invitrogen, USA) for each 50-mg sample according to the producer's instructions (Invitrogen, Carlsbad, USA). RNA quantity was measured using NanoDrop spectrophotometry and purity through 260/280 nm absorption ratio 1.80: 2.00. High-capacity cDNA reverse transcription kit (Invitrogen, USA) was used for reverse transcription according to kit protocol and following the same procedures detailed in our previous work (Elabd et al. 2016). Primers were provided by Metabion (Germany) (Table 2). Stratagene MX3005P was used in performing the RT-PCR reaction. The RT-PCR run was adjusted to include 1 cycle of 94 °C for 15 min and 40 cycles of 94 °C for 5 min, 62 °C for 30s, and 72 °C for 30s. The relative expression of each gene was calculated using the "ΔΔCt" method (Yuan et al. 2006).

Statistical analysis

Significant differences between groups were determined using one-way analysis of variance (ANOVA) and Duncan's multiple range tests through the Statistical Package for the Social Sciences (SPSS) software (v# 22.0). Values are expressed as means ± standard error and a value of $P < 0.05$ was regarded as significant.

Results

Growth performance

Growth performance parameters (SGR%, FCR, and LGR %) were ($P < 0.05$) significantly improved in

Table 2 Primer sequences of selected genes used in the gene expression study in Nile tilapia *O. niloticus*

Gene of interest	Primer sequences	Reference
EF-1α	CCTTCAACGCTCAGGTCATC TGTGGGCAGTGTGGCAATC	(Gröner et al. 2015)
IL1B	GCTGGAGAGTGCTGTGGAAGAACATATAG CCTGGAGCATCATGGCGTG	(Castro et al. 2011)
Hsp70	CTCCTGTGTGGGGGTTTTCC TTTGGGCTTCCCTCCGTCTG	(Shi et al. 2015)

SPNP groups compared with the control, with the most ($P < 0.05$) significant results in the 0.25% SPNP group at the end of the feeding trial (Table 3).

Immunological parameters

Lysozyme activity

SPNP incorporation revealed the most ($P < 0.05$) marked elevation in lysozyme activity for the 0.25% (w/w) SPNP group at the end of feeding trial (before exposure to any stress) (5.9 ± 0.0 U/L) and after exposure to cold stress (5.7 ± 0.05 U/L) and for the 0.5% (w/w) SPNP group (5.7 ± 0.0 U/L) after exposure to hypoxia stress over the control group (2.2 ± 0.05 U/L) (Fig. 1A).

Nitric oxide

Fish responded to stressors with elevated NO readings and SPNP ($P < 0.05$) markedly decreased those levels, particularly 0.5% SPNP (19.0 ± 0.0 umol/L) after exposure to hypoxia stress compared with the control (53.1 ± 0.02 umol/L) (Fig. 1B).

Antioxidant enzyme assays

Superoxide dismutase activity

The 0.25% SPNP efficiently mitigated the hypoxia stress ($P < 0.05$) and significantly elevated the SOD activity (46.7 ± 0.02 U/g tissue) that was decreased (39.7 ± 0.05 U/g tissue) after exposure to hypoxia stress. However, SPNP did not show a significant increase before and after cold stress compared with the control (Fig. 2A).

Catalase activity

The 0.5% SPNP-incorporated group was able to overcome the hypoxia stress and decrease (9.1 ± 0.0 U/g tissue) the elevated levels of CAT activity caused by the stress (36.1 ± 0.05 U/g tissue). SPNP incorporation did not reveal a ($P < 0.05$) significant enhancement in CAT activity compared with the control at before stress exposure or after exposure to cold challenge (Fig. 2B).

Glutathione peroxidase activity

Dietary incorporation with 0.25% SPNP showed the most ($P < 0.05$) marked increase (38.0 ± 0.0 U/g tissue) in GPx activity after the feeding trial and before exposure to any stress (Fig. 2C). Exposure to both hypoxia and cold increased GPx activity (28.6 ± 0.02 and 28.9 ± 0.0 U/g tissue), and SPNP supplementation was able to mitigate those stressors through decreasing GPx-elevated activity, with the ($P < 0.05$) best results in the 0.25% SPNP group after exposure to cold stress (14 ± 0.0 U/g tissue) and in the 0.5% SPNP group after exposure to hypoxia stress (12.2 ± 0.02 U/g tissue) (Fig. 2C).

Biochemical assays

Adding 0.25% SPNP to Nile tilapia diets showed the highest significant decrease ($P < 0.05$) in both ALT (16.9 ± 0.0 U/g tissue) and AST (16.0 ± 0.02 U/g tissue) activities at the end of the feeding trial and before stress exposure (Fig. 3A and B). The 0.25% SPNP group also revealed a significant decrease in AST activity after hypoxia stress (19.5 ± 0.0 U/g tissue) over the control (25.1 ± 0.02 U/g tissue) (Fig. 3B).

The SPNP groups showed a marked decrease ($P < 0.05$) in glucose concentration after exposure to cold compared with control groups (216.3 ± 0.02 mg/dl)

Table 3 Growth performance parameters of *O. niloticus* fed with diets incorporated with 0, 0.25, and 0.50% SPNP for 30 days after exposure to cold and hypoxia challenges

SPNP %/kg feed	Initial wt (g)	Final wt (g)	Initial length (cm)	Final length (cm)	SGR (%)	FCR	LGR (%)
0	26 ± 0.3	28 ± 0.6	10 ± 0.5	11 ± 0.4	0.14 ± 0.03	19.4 ± 0.5	14.3 ± 0.5
0. 25	26 ± 0.3	41 ± 0.4*	10 ± 0.5	12 ± 0.7*	0.60 ± 0.06*	3.2 ± 0.3*	26.9 ± 0.05*
0.50	26 ± 0.3	39 ± 0.6*	10 ± 0.5	12 ± 0.1	0.60 ± 0.11*	3.8 ± 0.4*	21.1 ± 0.07

Values are expressed as mean value ($n = 30$) ± SEM. Mean values showing asterisk (*) are significantly different ($P < 0.05$)

SGR specific growth rate, *LGR* length gain rate, *FCR* feed conversion ratio

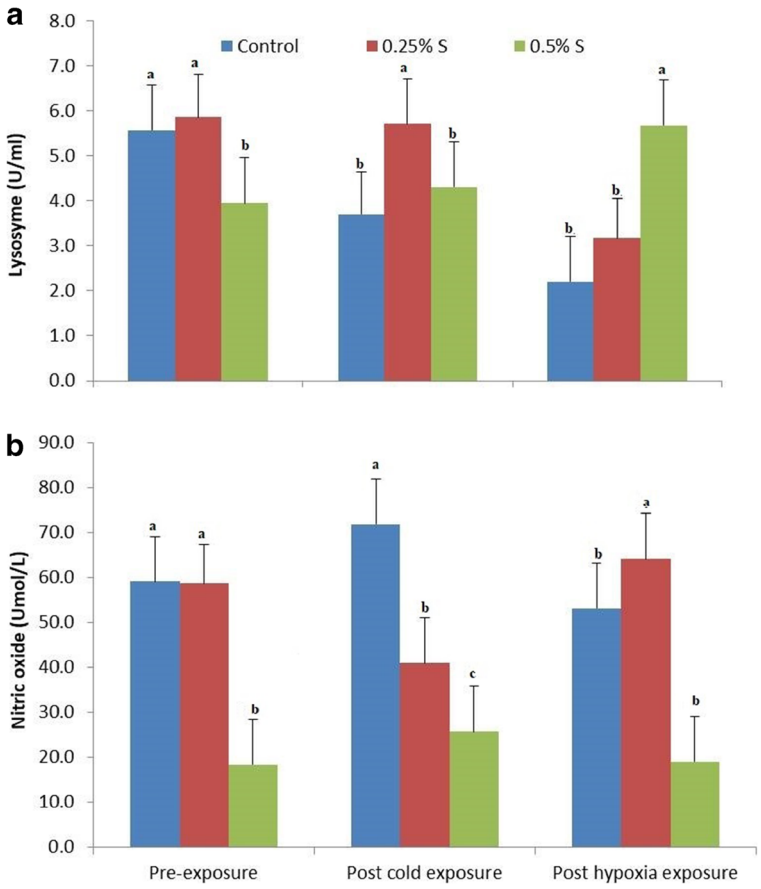

Fig. 1 Lysozyme and nitric oxide activities in Nile tilapia (*O. niloticus*) fed with (0.25 and 0.5%) SPNP-incorporated diets at the end of feeding trial and at post-exposure to cold and hypoxia challenges. Data is presented as mean ± SEM ($n = 9$). Values with different letter superscripts are significantly different ($P < 0.05$), depending on the compared control groups

(Fig. 4A) and in cortisol level before and after exposure to hypoxia. The most significant decrease in cortisol concentration was for the group receiving 0.25% SPNP at pre-exposure (10 ± 0.02 mg/dl) and for 0.5% SPNP at post-exposure to cold stress (5.8 ± 0.0 mg/dl) (Fig. 4B).

Digestive enzymes

Dietary SPNP ($P < 0.05$) enhanced the secretion of digestive enzymes (lipase and amylase) compared with the control group after exposure to the hypoxia challenge and 0.5% SPNP showed the highest enzyme values (7.2 ± 0.0 and 167.4 ± 0.02 U/L) (Fig. 5A and B).

Gene expression

Groups fed with 0.5% SPNP-incorporated diets showed marked upregulation ($P < 0.05$) in the expression of the Interleukin-1β (*IL-1β*) gene throughout the entire experiment (Fig. 6A) and in the expression of heat shock protein 70 (HSP70) gene after exposure to cold stress (Fig. 6B).

Challenge test with *A. veronii*

O. niloticus challenged with *A. veronii* showed loss of appetite, detached scales, ascitis, darkness of skin, and hemorrhagic patches all over the body. In addition, congestion of all internal organs (especially the kidney, liver, and spleen) was recorded, particularly in the control group. The SPNP-incorporated group showed 100% protection without any recorded mortalities compared with the control group, which showed 50% (Fig. 7).

Discussion

The current study concentrates on evaluating the effect of incorporating Nile tilapia diets with *Spirulina platensis* nanoparticles (SPNP). The incorporation of diets with SPNP ($P < 0.05$)

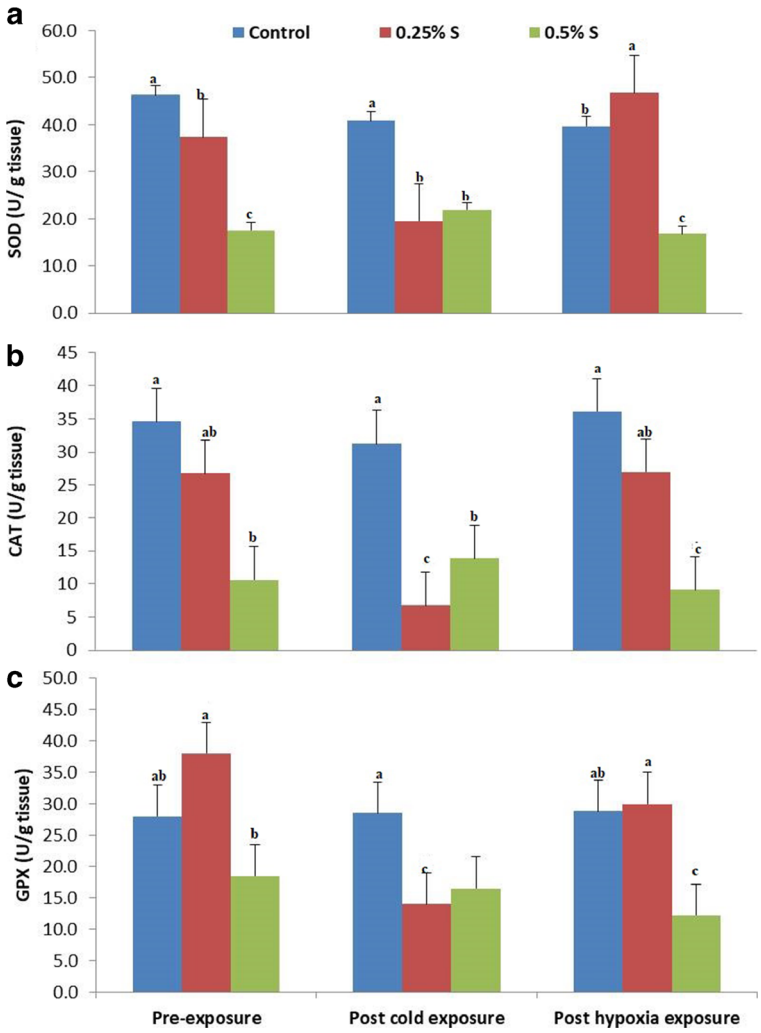

Fig. 2 Antioxidants SOD (A), CAT (B), and GPx (C) activities in the Nile tilapia's liver after feeding with (0.25 and 0.5%) SPN-supplemented diets before and after exposure to cold and hypoxia challenges. Data is presented as mean ± SEM ($n = 9$). Values marked with different letter superscripts are significantly different ($P < 0.05$)

significantly enhanced calculated growth parameters, with the most promising results for the 0.5% SPNP group. Meanwhile, Yu et al. (2018) and Adel et al. (2016) reported improved growth (WGR and SGR) of *P. leopardus* and of juvenile great sturgeon *Huso huso Linnaeus*, respectively, for the 10% *S. platensis* group. On the same instance, Amer (2016) revealed that the group supplemented with 1% *S. Platensis* had higher body weight than the control group, but it did not improve other growth parameters and took longer time to reach this effect. This comparison shows that SPNP is more efficient than its traditional form because the SPNP stays in the blood stream for long periods, increasing its availability and effectiveness (Nair et al. 2016; Hill and Li 2017). SPNP incorporation is more economical than traditional forms, as it requires the usage of smaller amounts of *Spirulina platensis*, which in this case was around only 7.5 g to reach the desired concentrations of SPNP all over the experimental period and costs less than $1 USD. Positive results of SPNP can be attributed to its high content from essential fatty acids, vitamins, and minerals. In addition, it has the ability to improve digestion and digestive enzyme activity, which in turn will improve growth performance (Promya and Chitmanat 2011; Ibrahem et al. 2013; Abdelkhalek et al. 2017).

Lysozyme activity is one of the most important immune response indicators (Magnadóttir 2006). Nitric oxide also plays a very important role in immune defense mechanisms (Villamil et al. 2002). Data of the current study indicates that the nonspecific immune

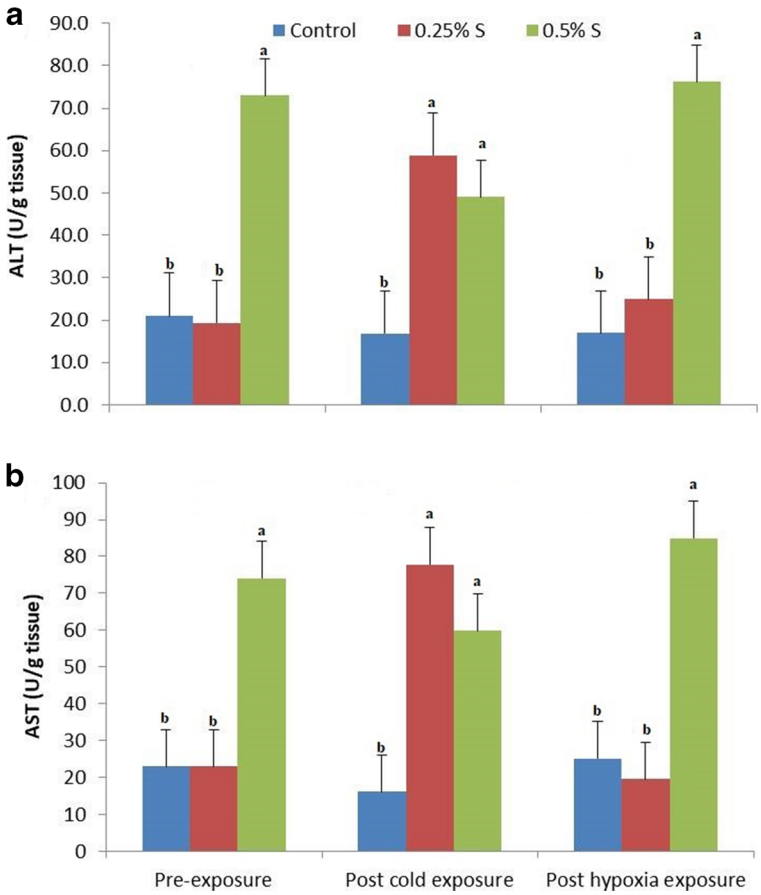

Fig. 3 Effect of 0.25 and 0.5% SPNP-supplemented diets on ALT (A) and AST (B) in the liver of Nile tilapia at pre- and post-exposure to cold and hypoxia challenges. Data is presented as mean ± SEM ($n = 9$). Values showing different letter superscripts are different significantly ($P < 0.05$)

parameters, especially lysozyme activity and nitric oxide level, were significantly higher ($P < 0.05$) for the 0.5% SPNP group after exposure to hypoxia stress compared with the control dose. In addition, the 0.25% SPNP group revealed a significant increase in lysozyme activity at the end of the feeding trial and after exposure to cold challenge, compared with the control. This increase could be related to the ability of *Spirulina* to enhance the immune system through improving phagocytic activities (Mahmoud et al. 2017). Similarly, previous studies reported the ability of *S. platensis* to increase lysozyme activity in Nile tilapia (Amer 2016; Mahmoud et al. 2017; Yilmaz 2019), Coral trout *Plectropomus leopardus* (Yu et al. 2018), and Carp *Cyprinus carpio* (Khalil et al. 2017).

Superoxide dismutase, catalase, and glutathione peroxidase are known as master antioxidative enzymes that may clean reactive oxygen species (ROS) and scavenge free harmful ROS (Madeira et al. 2013; Somogyi et al. 2007). In this study, the 0.5% SPNP showed the best ($P < 0.05$) results in mitigating elevated levels of SOD, CAT, and GPx, as it showed a significant decrease in those elevated levels especially after exposure to hypoxia stress. In the same instance, Mahmoud et al. (2017) and Khalil et al. (2017) reported that CAT, SOD, and GPX activities declined with the increase of dietary *S. platensis* incorporation in Nile tilapia and carp, respectively. Also, Amer (2016) showed that *S. Platensis* significantly decreased ($P < 0.05$) malondialdehyde compared with the control group in Nile tilapia. Those activities of *Spirulina* could be endorsed to its hepatoprotective effects (Mahmoud et al. 2018) and content of carotenoids that possess great antioxidant activity (Jensen et al. 1998; Hu et al. 2008), and of phytopigments as phycobilins, xanthophylls, and phycocyanin antioxidant activity (Bermejo et al. 2008).

Biochemical assessments are important in the evaluation of fish health (Mahmoud et al. 2018). Current study's results showed that 0.25% SPNP

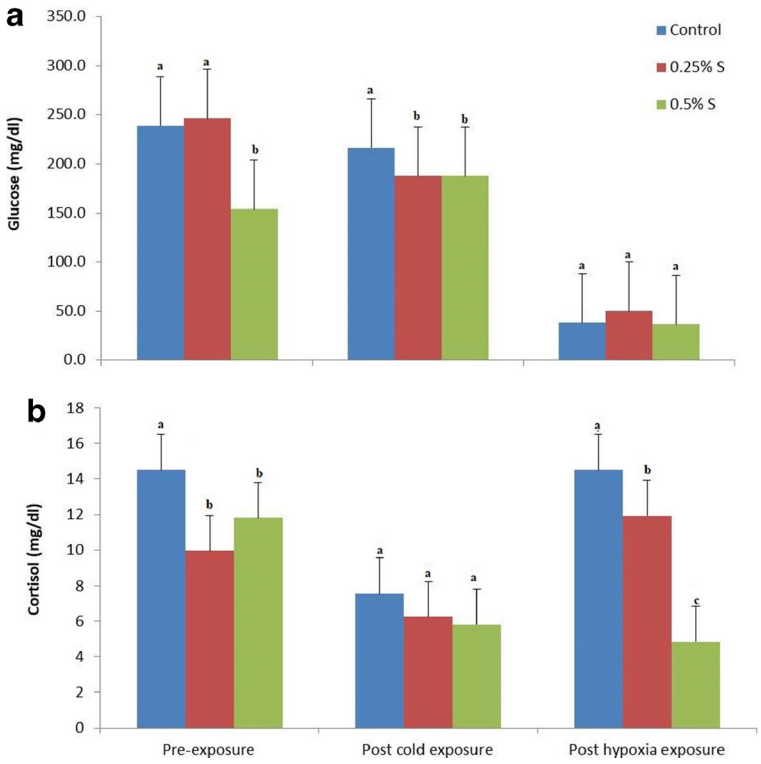

Fig. 4 Effect of 0.25 and 0.5% SPNP-supplemented diets on plasma glucose (A) and cortisol (B) of Nile tilapia (*O. niloticus*) at pre- and post-exposure to cold and hypoxia challenges. Data is presented as mean ± SEM ($n = 9$). Values showing different letter superscripts are different significantly ($P < 0.05$)

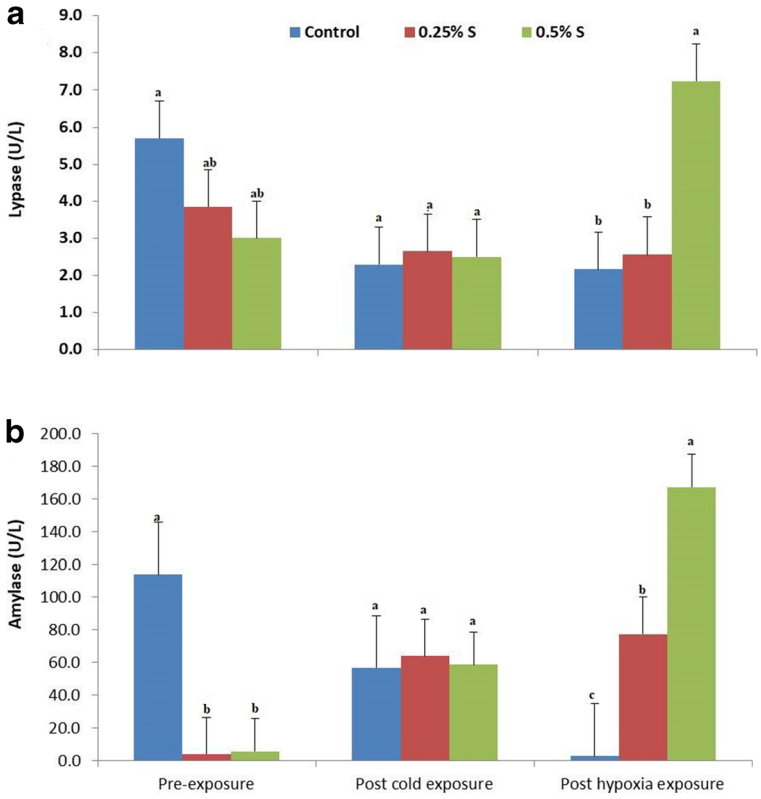

Fig. 5 Digestive enzyme (lipase and amylase) activities in plasma in Nile tilapia (*O. niloticus*) fed with (0.25 and 0.5%) SPNP-incorporated diets at the end of feeding trial and at post-exposure to cold and hypoxia challenges. Data is presented as mean ± SEM ($n = 9$). Values marked with letter superscripts are different significantly ($P < 0.05$)

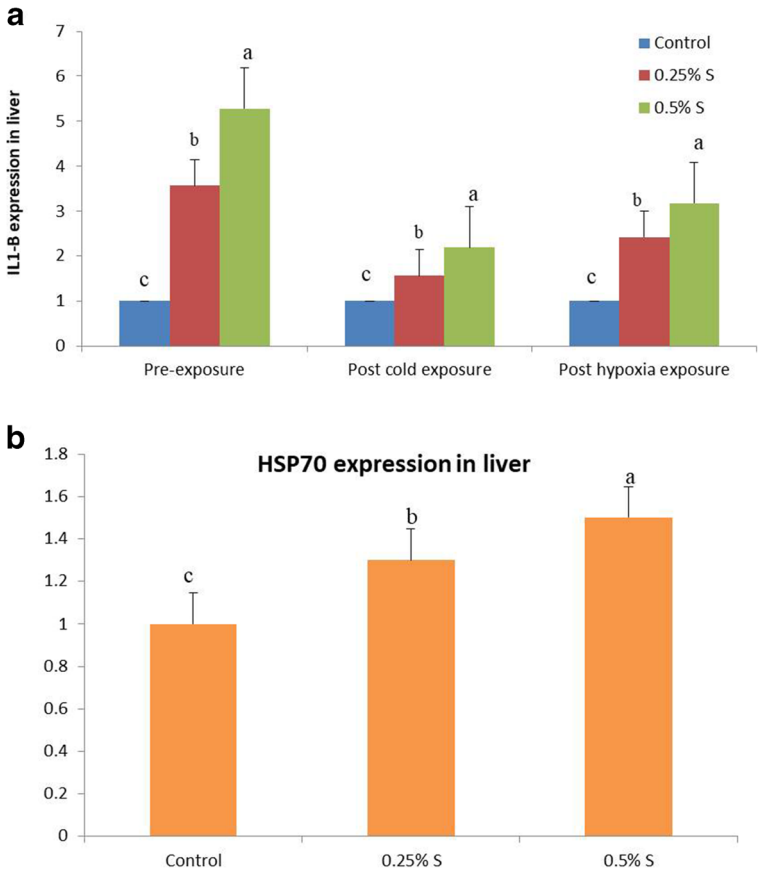

Fig. 6 Expression levels of *IL-1β* (A) and HSP70 (B) genes in Nile tilapia (*O. niloticus*) fed with (0.25 and 0.5%) SPNP-incorporated diets at the end of feeding trial and at post-exposure to cold and hypoxia challenges. Data is presented as mean ± SEM ($n = 9$). Values with different letter superscripts are different significantly ($P < 0.05$)

supplementation resulted in the most marked decrease ($P < 0.05$) in ALT and AST activities at the end of the feeding trial and before stress exposure in treated groups, compared with the control. These findings might be due to antioxidant activity of *Spirulina* (Bermejo et al. 2008; Mahmoud et al. 2018). The same results were reported by Mahmoud et al. (2017, 2018), who recorded a significant decrease in the *S. platensis*-incorporated group over the controls in Nile tilapia. In addition, plasma cortisol and glucose levels in the present study revealed the most marked decrease in the 0.25% SPNP group throughout the experiment compared with the control. Plasma cortisol and glucose levels are often known to be indicators of stress conditions in aquaculture (Barton and Iwama 1991). The ability of SPNP to decrease those levels can be possibly clarified in that *S. platensis* has the ability to bring down cholesterol levels through its active components (Ponce-Canchihuamán et al. 2010; Mahmoud et al. 2017) and its hepatoprotective effects (Mahmoud et al. 2018). The same results were also reported by Elabd et al. (2017), Mahmoud et al. (2018), and Yu et al. (2018).

The current study indicated that dietary SPNP supplementation in Nile tilapia diets enhanced digestion and digestibility, and that was reflected by ($P < 0.05$) significantly high digestive enzyme activities (lipase and amylase), compared with the control group after exposure to both cold and hypoxia challenges. This can be related to the potency of *S. platensis* to enhance flora occupying the intestine and digestive enzymes, which will, in turn, enhance growth (Dawood and Koshio 2016). Likewise, Yu et al. (2018) recorded enhanced digestion and digestibility in coral trout *Plectropomus leopardus* for the *S. platensis*-incorporated groups. Also, Abdel-Tawwab et al. (2018) reported high activities of digestive enzymes in *O. niloticus* receiving cinnamon nanoparticles.

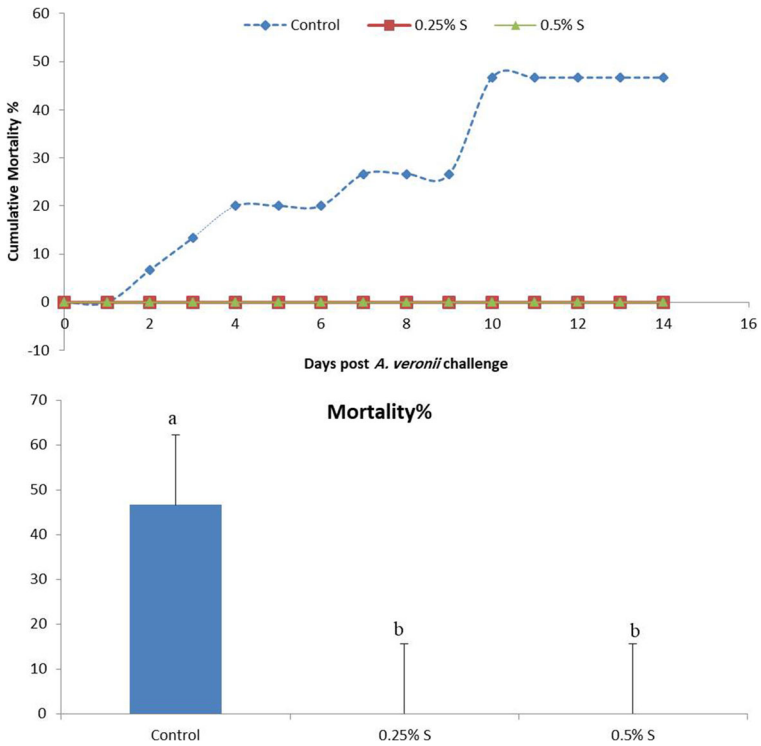

Fig. 7 Cumulative mortality rate of *Oreochromis niloticus* receiving 0.25 and 0.5% SPNP-incorporated diets after the 30-day feeding trial and challenged with pathogenic *A. veronii* for 15 days. Values marked with letter superscripts are different significantly ($P < 0.05$)

Gene expression study revealed that SPNP effectively upregulated the expression of the *IL-1β* gene throughout the entire experiment and upregulated the expression of the HSP70 gene after exposure to cold stress compared with the control group; those results come in accordance with immune response data indicating that those genes could be used as markers for stress response in *O. niloticus*. Interleukin-1 beta is an anti-inflammatory cytokine that induces immune response (Corripio-Miyar et al. 2007). HSP70 gene could be used as an indicator for stress conditions (Elabd et al. 2017). In the same instance, Elabd et al. (2017), Reda et al. (2018), and Yilmaz (2019) showed that dietary incorporation with different phyto-therapies positively upregulated the expression of *IL-1β* HSP70 genes. This can be caused by the immunostimulating properties of *S. platensis* (Mahmoud et al. 2017).

Recently, *S. platensis* dietary supplementations showed an increased survival rate in the case of a challenge with *Vibrio harveyi* (Yu et al. 2018), with *Pseudomonas fluorescens* (Mahmoud et al. 2018) and with *Aeromonas hydrophila* (Cao et al. 2018). To the best of our knowledge, there were no previous studies on the effect of *Spirulina* nanoparticles on the disease resistance of Nile tilapia challenged with *A. veronii* infection. In this study, the survival rate against *A. veronii* was 100% in the SPNP-supplemented groups. Similarly, fish fed with different supplements were found to be better protected than those of the control group (Reda et al. 2018; Yilmaz 2019). This could be due to the immunostimulating properties of dietary *Spirulina* caused by its content of different bioactive components such as β-carotene and phycocyanin (Cao et al. 2018).

In conclusion, the present study showed an improved effect of *Spirulina platensis* nanoparticles (SPNP) on growth, immune response, antioxidants, digestion, expression of related genes, and resistance to *A. veronii* infection in *O. niloticus*. Thus, SPNP can be economically used for mitigating hypoxia and cold stress conditions and controlling *A. veronii* infection in tilapia with improving growth, digestion, and immune and antioxidative responses.

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Compliance with ethical standards

Conflicts of interest All authors declare that they do not have any conflict of interest.

Ethics approval The current study was performed in agreement with the approved guidelines for the Use of Laboratory Animals by the Experimental Animal Use Committee, Benha University, Qalyubia, Egypt.

References

Abdelkhalek NKM, Eissa IAM, Ahmed E et al (2017) Protective role of dietary Spirulina platensis against diazinon-induced oxidative damage in Nile tilapia; Oreochromis niloticus. Environ Toxicol Pharmacol 54:99–104. https://doi.org/10.1016/j.etap.2017.07.002

Abdel-Tawwab M, Samir F, Abd El-Naby AS, Monier MN (2018) Antioxidative and immunostimulatory effect of dietary cinnamon nanoparticles on the performance of Nile tilapia, Oreochromis niloticus (L.) and its susceptibility to hypoxia stress and Aeromonas hydrophila infection. Fish Shellfish Immunol 74:19–25. https://doi.org/10.1016/j.fsi.2017.12.033

Adel M, Yeganeh S, Dadar M, Sakai M, Dawood MAO (2016) Effects of dietary Spirulina platensis on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (Huso huso Linnaeus, 1754). Fish Shellfish Immun 56:436–444

Aebi H (1984) [13] Catalase in vitro, in: Methods in Enzymology, Oxygen Radicals in Biological Systems. Academic Press, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Aly SM, Al Zohairy MA, Rahmani AH, FathiM, Atti NM (2016) Trials to improve the response of *Orechromis niloticus* to *Aeromonas hydrophila* vaccine using immunostimulants (garlic, Echinacea) and probiotics (Organic GreenTM and Vet-YeastTM). Afr J Biotechnol 15(21):989–994. https://doi.org/10.5897/AJB2015.15155

Amer S (2016) Effect of Spirulina platensis as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile tilapia (Oreochromis niloticus). Benha Vet Med J 30(1):1–10. https://doi.org/10.21608/bvmj.2016.31332

Anderson DP, Dixon OW, Simon RC (1980) Fish biologics: vaccine standards for sport fisheries and commercial aquaculture. Dev Biol Stand 45:157–162

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3–26. https://doi.org/10.1016/0959-8030(91)90019-G

Bermejo P, Piñero E, Villar ÁM (2008) Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulina platensis. Food Chem 110:436–445. https://doi.org/10.1016/j.foodchem.2008.02.021

Cao S, Zhang P, Zou T, Fei S, Han D, Jin J, Liu H, Yang Y, Zhu X, Xie S (2018) Replacement of fishmeal by spirulina Arthrospira platensis affects growth, immune related-gene expression in Gibel carp (Carassius auratus gibelio var. CAS III), and its challenge against Aeromonas hydrophila infection. Fish Shellfish Immunol 79:265–273. https://doi.org/10.1016/j.fsi.2018.05.022

Castro R, Zou J, Secombes CJ, Martin SAM (2011) Cortisol modulates the induction of inflammatory gene expression in a rainbow trout macrophage cell line. Fish Shellfish Immunol 30:215–223. https://doi.org/10.1016/j.fsi.2010.10.010

Corripio-Miyar Y, Bird S, Tsamopoulos K, Secombes CJ (2007) Cloning and expression analysis of two pro-inflammatory cytokines, IL-1 beta and IL-8, in haddock (Melanogrammus aeglefinus). Mol Immunol 44:1361–1373. https://doi.org/10.1016/j.molimm.2006.05.010

Dawood MAO, Koshio S (2016) Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture 454:243–251. https://doi.org/10.1016/j.aquaculture.2015.12.033

Dong HT, Techatanakitarnan C, Jindakittikul P, Thaiprayoon A, Taengphu S, Charoensapsri W, Khunrae P, Rattanarojpong T, Senapin S (2017) Aeromonas jandaei and Aeromonas veronii caused disease and mortality in Nile tilapia, Oreochromis niloticus (L.). J Fish Dis 40:1395–1403. https://doi.org/10.1111/jfd.12617

El Latif AMA, Elabd H, Amin A et al (2019) High mortalities caused by Aeromonas veronii: identification, pathogenicity, and histopathological studies in Oreochromis niloticus. Aquac Int 27:1725–1737. https://doi.org/10.1007/s10499-019-00429-8

Elabd H, Wang H-P, Shaheen A, Yao H, Abbass A (2016) Feeding Glycyrrhiza glabra (liquorice) and Astragalus membranaceus (AM) alters innate immune and physiological responses in yellow perch (Perca flavescens). Fish Shellfish Immunol 54:374–384. https://doi.org/10.1016/j.fsi.2016.04.024

Elabd H, Wang H-P, Shaheen A et al (2017) Anti-oxidative effects of some dietary supplements on Yellow perch (Perca flavescens) exposed to different physical stressors. Aquac Rep 8:21–30. https://doi.org/10.1016/j.aqrep.2017.09.002

FAO (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all, Rome, p 200

Fitzsimmons K (2016) Supply and demand in global tilapia markets, 2015. Aquaculture 2016, Las Vegas, pp 23–26. https://doi.org/10.13140/rg.2.1.2848.9448

Gröner F, Ziková A, Kloas W (2015) Effects of the pharmaceuticals diclofenac and metoprolol on gene expression levels of enzymes of biotransformation, excretion pathways and estrogenicity in primary hepatocytes of Nile tilapia (Oreochromis niloticus). Comp Biochem Physiol Toxicol Pharmacol CBP 167:51–57. https://doi.org/10.1016/j.cbpc.2014.09.003

Güroy B, Şahin İ, Mantoğlu S, Kayalı S (2012) Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid Pseudotropheus acei. Aquac Int 20:869–878. https://doi.org/10.1007/s10499-012-9512-x

Hill EK, Li J (2017) Current and future prospects for nanotechnology in animal production. J Anim Sci Biotechnol 8:26. https://doi.org/10.1186/s40104-017-0157-5

Hu C-C, Lin J-T, Lu F-J, Chou FP, Yang DJ (2008) Determination of carotenoids in Dunaliella salina cultivated in Taiwan and antioxidant capacity of the algal carotenoid extract. Food Chem 109:439–446. https://doi.org/10.1016/j.foodchem.2007.12.043

Huang X-J, Choi Y-K, Im H-S, Yarimaga O, Yoon E, Kim H-S (2006) Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors 6:756–782

Ibrahem M, Mohamed M, Ibrahim M (2013) The role of Spirulina platensis (Arthrospira platensis) in growth and immunity of Nile tilapia (Oreochromis niloticus) and its resistance to bacterial infection. J Agric Sci 5:p109. https://doi.org/10.5539/jas.v5n6p109

Jensen C, Birk E, Jokumsen A, Skibsted LH, Bertelsen G (1998) Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (Oncorhynchus mykiss) and during chill storage of smoked trout. Z Für Leb -Forsch A 207:189–196. https://doi.org/10.1007/s002170050317

Khalil SR, Reda RM, Awad A (2017) Efficacy of Spirulina platensis diet supplements on disease resistance and immune-related gene expression in Cyprinus carpio L. exposed to herbicide atrazine. Fish Shellfish Immunol 67:119–128. https://doi.org/10.1016/j.fsi.2017.05.065

Klemm DJ, Stober QJ, Lazorchak JM (1993) Fish field and laboratory methods for evaluating the biological integrity of surface waters. Environmental Monitoring Systems Laboratory-Cincinnati, Office of Modeling, Monitoring Systems, and Quality Assurance, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati

Liu Z, Que S, Xu J, Peng T (2014) Alanine aminotransferase-old biomarker and new concept: a review. Int J Med Sci 11:925–935. https://doi.org/10.7150/ijms.8951

Madeira D, Narciso L, Cabral HN, Vinagre C, Diniz MS (2013) Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comp Biochem Physiol A Mol Integr Physiol 166:237–243. https://doi.org/10.1016/j.cbpa.2013.06.008

Magnadóttir B (2006) Innate immunity of fish (overview). Fish Shellfish Immunol 20:137–151. https://doi.org/10.1016/j.fsi.2004.09.006

Mahmoud HK, Al-Sagheer AA, Reda FM et al (2017) Dietary curcumin supplement influence on growth, immunity, antioxidant status, and resistance to Aeromonas hydrophila in Oreochromis niloticus. Aquaculture 475:16–23. https://doi.org/10.1016/j.aquaculture.2017.03.043

Mahmoud MMA, El-Lamie MMM, Kilany OE, Dessouki AA (2018) Spirulina (Arthrospira platensis) supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (Oreochromis niloticus) challenged with Pseudomonas fluorescens. Fish Shellfish Immunol 72:291–300. https://doi.org/10.1016/j.fsi.2017.11.006

Nair M, Jayant RD, Kaushik A, Sagar V (2016) Getting into the brain: potential of nanotechnology in the management of NeuroAIDS. Adv Drug Deliv Rev 103:202–217. https://doi.org/10.1016/j.addr.2016.02.008

Palmegiano GB, Gai F, Daprà F, Gasco L, Pazzaglia M, Peiretti PG (2008) Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (Acipenser transmontanus) fingerlings. Aquac Res 39:587–595. https://doi.org/10.1111/j.1365-2109.2008.01914.x

Ponce-Canchihuamán JC, Pérez-Méndez O, Hernández-Muñoz R, Torres-Durán PV, Juárez-Oropeza MA (2010) Protective effects of Spirulina maxima on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. Lipids Health Dis 9:35. https://doi.org/10.1186/1476-511X-9-35

Promya J, Chitmanat C (2011) The effects of Spirulina platensis and Cladophora algae on the growth performance, meat quality and immunity stimulating capacity of the African sharptooth catfish (Clarias gariepinus). Int J Agric Biol 13:77–82

Reda RM, Selim KM, Mahmoud R, El-Araby IE (2018) Effect of dietary yeast nucleotide on antioxidant activity, non-specific immunity, intestinal cytokines, and disease resistance in Nile tilapia. Fish Shellfish Immunol 80:281–290. https://doi.org/10.1016/j.fsi.2018.06.016

Shaheen AA (2013) An industry assessment of tilapia farming in Egypt. African Union – Inter-African Bureau for Animal Resources (AU-IBAR)

Shi G-C, Dong X-H, Chen G, Tan BP, Yang QH, Chi SY, Liu HY (2015) Physiological responses and HSP70 mRNA expression of GIFT strain of Nile tilapia (Oreochromis niloticus) under cold stress. Aquac Res 46:658–668. https://doi.org/10.1111/are.12212

Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy G (2007) Antioxidant measurements. Physiol Meas 28:R41–R55. https://doi.org/10.1088/0967-3334/28/4/R01

Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E (2014) Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol 40:40–48. https://doi.org/10.1016/j.fsi.2014.06.016

Villamil L, Tafalla C, Figueras A, Novoa B (2002) Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (Scophthalmus maximus). Clin Diagn Lab Immunol 9:1318–1323. https://doi.org/10.1128/CDLI.9.6.1318-1323.2002

Yilmaz S (2019) Effects of dietary blackberry syrup supplement on growth performance, antioxidant, and immunological responses, and resistance of Nile tilapia, Oreochromis niloticus to Plesiomonas shigelloides. Fish Shellfish Immunol 84:1125–1133. https://doi.org/10.1016/j.fsi.2018.11.012

Yu W, Wen G, Lin H, Yang Y, Huang X, Zhou C, Zhang Z, Duan Y, Huang Z, Li T (2018) Effects of dietary Spirulina platensis on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and disease resistance of coral trout Plectropomus leopardus (Lacepede, 1802). Fish Shellfish Immunol 74:649–655. https://doi.org/10.1016/j.fsi.2018.01.024

Yuan JS, Reed A, Chen F, Stewart CN (2006) Statistical analysis of real-time PCR data. BMC Bioinformatics 7:85. https://doi.org/10.1186/1471-2105-7-85