DOI: 10.1002/vms3.490

ORIGINAL ARTICLE

WILEY

Trial for use nanoselenium particle with different dietary regime in *Oreochromis niloticus* and *Mugil cephalus* polyculture ponds: Growth efficiency, haematological, antioxidant, immunity and transcriptional analysis

Eman M. Moustafa[1] | Marwa F. Abd El-Kader[2] | Montaser M. Hassan[3] | Ahmed F. Fath El-Bab[4] | Amira Omar[1] | Foad Farrag[5] | Ahmed G. Gewida[6] | Mohamed F. Abd-Elghany[6] | Mustafa Shukry[7] | Rasha A. Alwakeel[7]

[1]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr el-Sheikh, Egypt

[2]Fish Diseases and Management, Sakha Aquaculture Research Unit, Central Lab for Aquaculture Research, A.R.C., Cairo, Egypt

[3]Department of Biology, College of Science, Taif University, Taif, Saudi Arabia

[4]Animal Production, Faculty of Agriculture, Damietta University, Damietta, Egypt

[5]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

[6]Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

[7]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

Correspondence
Eman M. Moustafa, Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt.
Email: emantarek2002@yahoo.com

Funding information
The current work was funded by Taif University Researchers Supporting Project number (TURSP-2020/119), Taif University, Taif, Saudi Arabia.

Abstract

Background: Fish farming is one of the most productive economies in the world. One of the essential goals in fish production is to minimize processing costs while maintaining and increasing the vital functions, weight and immunity of fish.

Objective: We conducted this study to explore nanoselenium (Nano-Se) particles in various feeding schemes.

Material and Method: Nano-Se particles incorporated in the basal diet at (0.5 mg/kg diet), and the fish was divided into six groups after adaptation as the follows: The first group was feed daily with a diet containing Nano-Se (0.5 mg/kg diet); the second group was exposed to a feeding programme in which it has day feeding followed by day of starvation with a diet containing Nano-Se (0.5 mg/kg diet); the third group was day feeding followed by 2 days of starvation; the fourth group served as a negative control group in which this group was continuous feeding with a basal diet without Nano-Se; the fifth group was day feeding with the basal diet followed by a day of starvation; and the sixth group was day feeding with basal diet followed by 2 days of starvation.

Result: Our result revealed that Group 2 showed significant improvement in haematological parameters, red blood cells and haemoglobin with a substantial increase in total protein ($p < 0.05$) as well as lysosomal and phagocytic activity with considerable upregulation of growth hormone and insulin growth factor 1 in addition to markedly increase in the pro-inflammatory cytokines. Finally, this study offers the first-time dietary regime with Nano-Se supplementation that saves the feeding cost and increases fish welfare and growth.

KEYWORDS

cytokines, growth markers genes, *Mugil cephalus*, Nano-Se, *Oreochromis niloticus*, performance

1 | INTRODUCTION

Understanding and implementing appropriate feeding strategies for nutrients can reduce waste and increase profit. In general, feed quality is essential for livestock farming. The feeding frequency dictates the time (feeding interval) between meals. The effects of interval feeding in fish depend on the species, sex, age and capacity. For compensatory growth and deposition of fat, for optimizing development and increasing feed performance, feeding intervals in favour of optimum digestibility and protein, tilapia were fed at intervals of 2–3 hr eat more than its volume of the stomach, so that some feed passes through the stomach without being digested (Riche, Oetker, et al., 2004). A feeding rate of one meal every 4–5 hr (three times a day) delivers optimal results (Riche, Haley, et al., 2004). Restricted feeding has lower costs, increased feeding performance, reduced carcass fat build-up and better water quality.

Several studies have been used to boost feed quality, such as gastric evacuation time that enriches the fish appetite and refeeding after starvation, enhancing feed utilization and development (Chan et al., 2008; Riche, Haley, et al., 2004). Feeding intervals have a precise impact on the water quality, fish well-being and immunity (Garcia & Villarroel, 2009; Lee et al., 2000). There was an optimum feeding frequency of twice daily for the larvae and juveniles of hybrid tilapia (Qiang et al., 2009) at the same time as six times per day has been described for juveniles (Tung & Shiau, 1991). The mixed-sex juveniles Nile tilapia have gained optimum growth when fed four times a day in brackish water (Daudpota et al., 2016) while providing six times per day was optimum for male juveniles a freshwater pond (Pouomogne & Ombredane, 2001). This dispute's outcomes are based on variations in experimental fish genetic origins, nutrition, age and culture conditions. The growth output of sex-reversed Nile tilapia has been affected by different feeding frequencies. Different feeding frequencies had a significant effect on the growth performance of sex-reversed Nile tilapia. Fish fed twice and three times a day showed higher growth rate due to the increased Chymotrypsin and other digestive enzymes (Thongprajukaew et al., 2017).

Tilapia is well suited to many environments and feeding plans (Byamungu et al., 2001), although increased feeding rates seem to confer more excellent disease resistance (Garcia et al., 2009). Grey mullets (Mugilidae family) play an essential role in the fish cultures globally, South East Asia, primarily the Mediterranean (Crosetti, 2015). Although mullets are the main significant farmed fish in many countries, no different feeds are offered (Crosetti, 2015). Mullets are very popular as food in Egypt. They have been the cornerstone of fish farming for centuries because their fries are different seasons of the year, available in millions in fresh and brackish water (Wassef et al., 2001).

Nanotechnologies in aquaculture provide a wide variety of applications, ranging from sterilizing pools, water treatment, identification and management of aquatic conditions, adequate supplies of nutrients and medicines (including hormones and vaccine, Huang et al., 2015). The interest in using nano-trace elements as animal feed supplements has recently increased due to increased bioavailability concerning inorganic salts. However, the use of high amounts of inorganic Se has posed environmental issues due to the high volume of faecal Se excretion (Dawood, Koshio, Zaineldin, Van Doan, Moustafa, et al., 2019). Nanoparticles are more accessible to the biological system in small quantities, decreasing feeding costs (Naderi et al., 2019). Trace minerals are essential in the nutrition and metabolism of many living species. Selenium (Se) is one of those constituencies. It plays a crucial part in antioxidant and disease resistance and growth (Suttle, 2010).

Selenium deficiency in the diet of fish led to an increase in the chance of disease, retardation of development and declined immunity (Jobling, 2012). Today, nanotechnology is used in aquaculture for many purposes due to its ability to increase feed absorption (El Basuini et al., 2017). Nanoselenium (Nano-Se) has recently been bioavailable in fish diets to improve the immune and antioxidant response (Saffari et al., 2018). Nanoparticles with a particular smaller dimension than a hundred nm give a more significant proportion of surface ions and physicochemical shifts. Nanoparticles may enter the body through gastrointestinal tract or another different way; inside the body, these nanoparticles will contact immune cells (Cupaioli et al., 2014). Selenium is a vital microelement essential for developing fish efficiency and health (Pacitti et al., 2016). Selenium nanoparticle has been utilized due to its high-level bioavailability and low malignancy when fed to fish in adequate quantities (Dawood, Koshio, Zaineldin, Van Doan, Moustafa, et al., 2019). The Nano-Se application had beneficial effects on many fish species' efficiency and wellness (Saffari et al., 2017). The current study aimed to assess the influence of feeding Nano-Se particles with different feeding and starvation regime in *Oreochromis niloticus* and *Mugil cephalus* growth performance, liver function and antioxidant activity (superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]). Besides, evaluate their effect on the haematological markers, phagocytic activity and lysozyme activity as well as the impact of Selenium nanoparticles on growth markers gene and cytokines gene expression.

2 | MATERIAL AND METHODS

2.1 | Experimental design

Fish were raised in standard concrete ponds (2 × 1 × 1 m) on a private farm located in Dakahlia, Egypt, eight *O. niloticus* fish and seven *M. cephalus* in 1 m with initial weight 27 ± 0.5 and 25 ± 1 g/fish, respectively. The fish were acclimated for 14 days before beginning the experiment; after that, the fish were distributed randomly into six groups (two replicates for each group). The first group was fed daily with a diet containing Nano-Se (0.5 mg/kg diet); the second group was exposed to a feeding programme in which it has day feeding followed by day of starvation with a diet containing Nano-Se (0.5 mg/kg diet) according to (Abd El-Kader et al., 2020); the third group was day feeding followed by 2 days of starvation; the fourth group served as a negative control group in which this group was continuous feeding with a basal diet without Nano-Se; the fifth group was day feeding with the basal diet followed by a day of starvation; and the sixth group was day feeding with basal diet followed by 2 days of starvation (see Figure S1).

Groups	Diet	Number of replicates	Feeding programme
Group 1	Basal diet + Nano-Se (0.5 mg/kg diet)	2	Continuous feeding
Group 2	Basal diet + Nano-Se (0.5 mg/kg diet)	2	Day feeding followed by day of starvation
Group 3	Basal diet + Nano-Se (0.5 mg/kg diet)	2	Day feeding followed by 2 days of starvation
Group 4	Basal diet only	2	Continuous feeding
Group 5	Basal diet only	2	Day feeding followed by day of starvation
Group 6	Basal diet only	2	Day feeding followed by 2 days of starvation

The fish in all the groups were fed at a rate of 3% of body weight-adjusted weekly according to weight. Each diet (29% protein) was provided to its respective pond according to the feeding programme for 12 weeks. After diet formation, the selenium ratio will be 0.06 mg/kg diet and a 0.58 mg/kg diet for the control and Nano-Se group. All fish have been carefully weighed in bulk weekly for growth and health tests. The water quality parameters were not significantly differentiated during the experimental period. The water's average temperature was 24.1 ± 0.3°C and dissolved O2 6.2 ± 0.42 mg/L; pH was 7.24–7.46, ammonia concentration 0.22–0.23 mg/L as assayed by DREL/2 HACH kits (Hach Co.) (see Table S1).

2.2 | Growth parameter

The following equations were used to calculate the weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) after fasting the fish for 24 hr. WG (WG %) = 100 × [final body weight (FBW, g) − initial body weight (IBW, g)]/IBW (g). Daily weight gain (DWG) = (mean final weigh − tcmean initial weight)/(days). SGR (%/day) = 100 × [ln FBW (g) − ln IBW (g)]/days. FCR = total dry feed intake (g)/[FBW (g) − IBW (g)].

2.3 | Sampling

All fish were anesthetically screened using MS222 150 mg/L (Argent Laboratories) at zero-days and at the end of the 12-week feeding experiment.

Each fish's weight was individually assessed. Blood samples were obtained using an anticoagulant syringe and without-anticoagulant syringe from four fish caudal blood vessels per pond, and serum obtained using 1968 *g* /15 min at 4°C centrifuges. The serum was kept at −20°C until further analysis.

2.4 | Haematology and biochemical parameters

After dissolution with Natt and Herrick's solution, a haemocytometer was used immediately with red blood cells (RBCs) as well as white blood cells (WBCs) (Houston, 1990). For WBCs and differential count were assayed according to (Jain, 1986; Lucky & Lucký, 1977), Hb concentration analysis following (Blaxhall & Daisley, 1973). Total protein, albumin, globulin, ALT and AST assessed using ready-made chemicals (kits) supplied by Spinreact Co., according to the manufacturer's instructions with an RA-50 chemistry analyzer (Bayer). Phagocytic activity is determined according to Kawahara et al. (1991). Phagocytic activity = macrophages containing yeast/total number of macrophages × 100; phagocytic index = number of cells phagocytized/number of phagocytic cells. The lysozyme activity was determined according to Parry et al. (1965).

2.5 | Antioxidants markers analysis

In the fish serum, the levels of SOD, CAT, GPx and malondialdehyde (MDA) were assessed using diagnostic reagent kits following the procedure for the manufacturer (Cusabio Biotech Co., Ltd).

2.6 | Gene expression

Analysis of mRNA expression for different genes (real-time polymerase chain reaction [RT-PCR]) and β-actin (an internal guide for normalizing data on gene expression) was performed using the primers shown in Table 1. Following the manufacturer's protocol, the total RNA was extracted from the liver samples using Trizol reagents Trizol (iNtRON Biotechnologie). With 2% agarose electrophoresis, the content of the extracted RNA was confirmed. Nanodrop (Quawell) determined the RNA concentration; 2 μg of total RNA was reverse transcribed using the manufacturer's cDNA synthesis kit (Bioline) as a guide. In 20-μl reaction mixtures containing 2 μl of cDNA, gene-specific primers (0.5 μM each) and SYBR 10 μl, real-time PCR amplifications were performed using the SensiFast SYBR Lo-Rox kit (Bioline). Thermal cycling conditions were initial denaturation at 95°C for 10 min, then 40 cycles at 95°C for 15 s and 60°C for 1 min. They double-checked the genes using ($2^{-\Delta\Delta CT}$) (Livak & Schmittgen, 2001).

2.7 | Data analysis

The tests of Shapiro–Wilk and Levene confirmed that the variance was normal distribution and homogeneous. All statistical differences were measured by the one-way analysis of variance (ANOVA) research (SPSS version 23; SPSS Inc.) and by Duncan as a post hoc test. Where there were differences between groups of study, they were accepted at $p < 0.05$. All data are displayed as of mean ± *SE*. Two-way ANOVA was used for gene expression analysis.

TABLE 1 Gene primer for real-time PCR

Target gene	Forward	Reverse	Accession number
β-actin (*Oreochromis niloticus*)	GTGCCCATCTACGAGGGTTA	CTCCTTAATGTCACGCACGA	Pang et al. (2013)
β-actin (Mugil)	TGCAGTCAACATCTGGAATC	ATTTTTGGCGCTTGACTCAG	Abdel-Mageid et al. (2020)
IL-1β (*O. niloticus*)	CTTCCCATAGACTCTGAGTAGCG	AAGGATGACGACAAGCCAAC	KF747686.1
IL-1β (Mugil)	GAGGAGCTTGGTGCAGAACA	CTTTGTTCGTCACCTCCTCCA	Abdel-Mageid et al. (2020)
IGF-1 (*O. niloticus*)	CACCCTCTCACTACTGCTGT	CACAGTACATCTCAAGGCGC	EU272149.1
IGF-1 (Mugil)	ACCTGATGAGTGGGAAGTGG	GCATCTCCGGCTCATCTTTG	AY772256.1
GH (*O. niloticus*)	CTGGTTGAGTCCTGGGAGTT	CAGGTGGTTAGTCGCATTGG	KT387598.1
GH (Mugil)	TGCTTCAAAAAGGACATGCA	GATGTTTGCAGGTTGAG AC	AF134605

Abbreviation: PCR, polymerase chain reaction; IL-1β, Interleukin 1 Beta; IGF-1, Insulin growth factor 1; GH, Growth hormone.

TABLE 2 Haematological and biochemical parameters of *Oreochromis niloticus* at zero day of experiment

	G1	G2	G3	G4	G5	G6	*SE*	*p* value
RBCs	2.125	2.11	2.065	2.085	2.055	2.025	0.04	0.351
Hb	6.33	6.32	6.255	6.315	6.235	6.265	0.05	0.524
PCV	20	20	19.5	20	19	19	0.64	0.424
MCV	94.13	94.75	94.435	95.94	92.475	93.84	2.5	0.84
MCH	29.79	29.955	30.305	30.29	30.34	30.94	0.39	0.209
MCHC	31.65	31.665	32.09	31.575	32.815	32.975	0.85	0.465
WBCs	10.24	9.67	10.11	10.22	10.20	10.22	0.21	0.319
Heterophil	1.44	1.16	1.42	1.33	1.23	1.38	1.9	0.413
Lymphocyte	7.83	7.78	7.84	7.92	8.16	7.87	1.4	0.124
Monocyte	0.82	0.68	0.76	0.72	0.72	0.77	0.4	0.212
Eosinophil	0.10	0.00	0.05	0.10	0.00	0.05	0.4	0.158
Basophil	0.05	0.05	0.05	0.16	0.10	0.16	0.64	0.424
Lysozyme	8.895	8.895	9.04	8.955	9.005	8.975	0.05	0.216
Phagocytic activity	9.985	10.025	10.005	9.44	9.795	9.94	0.29	0.423
Phagocytic index	0.84	0.935	0.97	0.89	0.98	0.855	0.03	0.15
Total protein	3.755	3.585	3.685	3.665	3.64	3.65	0.05	0.149
Albumin	1.53	1.465	1.54	1.51	1.51	1.52	0.02	0.273
Globulin	2.225	2.12	2.145	2.155	2.13	2.13	0.06	0.592
AST	29.2	28.87	28.57	28.82	28.535	28.88	0.41	0.631
ALT	26.995	26.52	26.8	27.425	27.035	26.755	0.64	0.795
MDA	18.57	20.46	19.205	19.17	19.325	19.075	0.58	0.176
GPx	12.985	13.41	13.515	13.61	13.215	13.59	0.19	0.99
CAT	10.615	10.475	10.57	10.665	10.475	10.505	0.04	0.226
SOD	10.19	10.225	10.295	10.3	10.36	10.3	0.09	0.554

Note: Values are expressed as means ± *SE*.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MDA, malondialdehyde; PCV, packed cell volume; RBCs, red blood cells; SOD, superoxide dismutase; WBCs, white blood cells.

3 | RESULTS

3.1 | Effect of Nano-Se on the haematological and biochemical parameter

There was no significant difference in haematological and biochemical parameters and no variation in protein content, liver enzymes, phagocytic index and lysosomal activity between different treated groups in day zero treatment in *O. niloticus* fish and Mugil fish, as shown in Tables 2 and 3.

After 12 weeks of treatment, our result showed that Group 2 (Nano-Se + day feed + day starvation), as well as Group 1 (continuous Nano-Se feeding), was significantly increased in packed cell volume and RBCs (Table 4) as well as lysosomal activity and total leukocytic count with decreased heterophile % concerning other treated groups of *O. niloticus* fish. In the same way, there was a markedly increase in lysosomal, phagocytic activity and index, as well as there was a significant increase in globulin and total protein concentration with a markedly decrease in MDA level with a substantial rise in GPx, CAT and SOD concerning other treated groups.

From the previous result, it is evident that the feeding regime of Nano-Se + day feed + day starvation revealed a marked improvement in either haematological, biochemical or antioxidant activity followed by continuous Nano-Se feeding regime. In the same context, Mugil fish Group 2 (Nano-Se + day feed + day starvation), as well as Group 1 (continuous Nano-Se feeding), significantly showed significant improvement in haematological and immunity (lysosomal, phagocytic activity, and index) concerning other treated groups which strength our obtained result concerning *O. niloticus* fish as shown in Table 5 with the significant increase in total protein and globulin with markedly decreased AST level.

3.2 | Effect of Nano-Se particles on the antioxidant activity

The antioxidants' profile in our result, as shown in Tables 2 and 3, revealed that there are no significant changes in the MDA level between different treated groups, *O. niloticus* and *M. cephalus*. In the same line, there is no significant alteration in the serum GPX, CAT

TABLE 3 Haematological and biochemical parameters of *Mugil cephalus* at zero day of experiment

	G1	G2	G3	G4	G5	G6	*SE*	*p* value
RBCs	3.08	3.075	3.165	3.12	3.09	3.095	0.04	0.394
Hb	9.41	9.455	9.595	9.4	9.525	9.495	0.08	0.352
PCV	29.5	29	30.5	30.5	29.5	30	0.57	0.182
MCV	95.78	94.31	96.36	97.75	95.465	96.93	1.02	0.126
MCH	30.555	30.75	30.32	30.13	30.825	30.675	0.26	0.2
MCHC	31.9	32.605	31.465	30.825	32.295	31.65	0.47	0.08
WBCs	12.5	11.9	11.8	12.1	11.9	12.0	0.3	0.519
Heterophil	1.9	1.8	1.8	1.9	1.8	2.1	1.6	0.673
Lymphocyte	9.5	9.0	9.0	9.1	9.2	8.6	2.04	0.323
Monocyte	1.0	0.8	0.8	1.0	0.8	1.0	0.707	0.158
Eosinophil	0.0	0.1	0.1	0.1	0.1	0.1	0.4	0.212
Basophil	0.1	0.1	0.1	0.2	0.1	0.1	0.7	0.833
Lysozyme	9.945	10.01	10.055	9.995	10.025	10.01	0.14	0.982
Phagocytic activity	10.89	11.01	10.97	11.19	10.96	11.1	0.08	0.08
Phagocytic index	0.925	1.06	0.985	1.065	0.835	1.035	0.06	0.048
Total protein	4.01	4.085	4.135	4.065	4.085	4.105	0.014	0.24
Albumin	1.74	1.7	1.745	1.735	1.705	1.775	0.035	0.394
Globulin	2.27	2.385	2.39	2.33	2.28	2.33	0.04	0.136
AST	20.085	19.775	20.06	19.71	19.795	19.975	0.127	0.098
ALT	22.97	23.125	22.92	23.12	23.305	22.935	0.19	0.44
MDA	21.52	22.045	21.725	22.45	21.795	22.16	0.19	0.129
GPx	13.825	13.935	14.005	14.055	13.925	14.04	0.11	0.396
CAT	9.94	9.95	9.965	9.9	9.9	9.895	0.08	0.921
SOD	9.93	10.09	10.06	10.095	10.01	10	0.06	0.223

Note: Values are expressed as means ± *SE*.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MDA, malondialdehyde; PCV, packed cell volume; RBCs, red blood cells; SOD, superoxide dismutase; WBCs, white blood cells.

TABLE 4 Effect of Nano-SE particles on the haematological and biochemical parameters of *Oreochromis niloticus* after 12 weeks of experiment

	G1	G2	G3	G4	G5	G6	*SE*	*p* value
RBCs	8.62^{a}	9.025^{a}	6.925^{c}	6.825^{c}	7.835^{b}	6.8^{c}	0.058	0
Hb	2.125	2.11	2.065	2.085	2.055	2.025	0.04	0.351
PCV	27^{a}	28^{a}	22^{c}	22^{c}	25^{b}	20.5^{c}	0.64	0
MCV	96.92	97.225	96.28	98.215	97.095	93.385	2.05	0.374
MCH	31.975	32.235	31.48	31.025	31.34	33.19	0.89	0.314
MCHC	30.955	31.335	30.305	30.47	30.425	30.975	0.36	0.149
WBCs	12.535^{b}	14.01^{a}	10.48^{d}	10.41^{d}	11.91^{c}	10.445^{d}	0.3	0
Heterophil	9.5^{e}	9.5^{e}	15^{b}	16^{a}	11^{d}	13.5^{c}	1.3	0.01
Lymphocyte	80.^{5a}	81.5^{a}	76.5^{b}	75^{c}	80.5^{a}	78^{b}	1.6	0.038
Monocyte	9	8	7.5	6.5	7.5	7	0.5	0.062
Eosinophil	0	0.5	1	1.5	0.5	1	0.5	0.182
Basophil	1	0.5	0	1	0.5	0.5	0.5	0.434
Lysozyme	11.655^{b}	12.115^{a}	9.745^{c}	9.335^{d}	9.915^{c}	8.995^{e}	0.25	0
Phagocytic activity	11.875^{b}	12.01^{a}	10.34^{c}	10.23^{c}	10.42^{c}	10.065^{d}	0.07	0
Phagocytic index	1.27^{a}	1.235^{a}	0.995^{c}	0.925^{c}	1.165^{b}	0.815^{d}	0.04	0
Total protein	4.075^{b}	4.19^{a}	3.77^{c}	3.715^{c}	3.815^{c}	3.72^{c}	0.04	0
Albumin	1.55	1.55	1.61	1.58	1.555	1.575	0.03	0.62
Globulin	2.525^{b}	2.64^{a}	2.16^{c}	2.135^{c}	2.26^{c}	2.145^{c}	0.03	0
AST	28.78^{b}	28.28^{b}	28.4^{b}	28.915^{a}	27.595^{c}	29.08^{a}	0.6	0.289
ALT	26.07^{b}	25.315^{b}	26.82^{b}	27.36^{a}	26.87^{a}	26.82^{a}	0.78	0.264
MDA	18.42^{d}	18.215^{d}	21.22^{c}	23.32^{b}	20.655^{c}	25.805^{a}	1.2	0.006
GPx	14.705^{b}	15.39^{a}	13.905^{d}	13.88^{d}	14.17^{c}	13.78^{d}	0.2	0.001
CAT	11.33^{b}	11.575^{a}	10.91^{c}	10.835^{c}	11.22^{b}	10.56^{d}	0.15	0.005
SOD	11.31^{b}	11.745^{a}	10.845^{c}	10.77^{d}	10.925^{c}	10.73^{d}	0.16^{d}	0.006

Note: Values are expressed as means ± *SE*. Different superscript letters indicate significant differences in the same column.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MDA, malondialdehyde; Nano-SE, nanoselenium; PCV, packed cell volume; RBCs, red blood cells; SOD, superoxide dismutase; WBCs, white blood cells.

and SOD levels between different treated groups at zero days of the experiment. After 12 weeks of treatment, MDA concentration was markedly decreased with significant elevation in the GPX, SOD and CAT levels in Nano-Se treated groups concerning other groups of *O. niloticus* and *M. cephalus* fish.

3.3 | Effect of Nano-Se particles on the gene expression analysis

Our obtained data showed in Figures 1 and 2 that there was a significant increase in IL1B, IL8, GH and IGF-1 in Group 2 (Nano-Se + day feed + day starvation) concerning other treated groups as well as between day zero and 12 weeks of treatment; in the same context, there was a significant improvement in gene expression of IL1B, IL8, GH and IGF-1 in Group 1 (continuous Nano-Se feeding) in comparison with the control groups regime and (Nano-Se + day feed + 2-day starvation). Groups 5 (control + day feed + day starvation) and 3 (Nano-Se + day feed + 2-day starvation) showed significant improvement in IL1B, IL8, GH and IGF-1 concerning day zero gene expression analysis in both *O. niloticus* and *M. cephalus* fish.

3.4 | Effect of Nano-Se particles on the growth parameters performance

As shown in Tables 6 and 7, there was a markedly increase in FBW, WG and daily WG and SGR in Group 2 treated fish (*O. niloticus* and *M. cephalus* fish) (Nano-Se + day feed + 1-day starvation) concerning other treated groups followed by improvement in growth parameters in Group 1, first feeding regime (continuous Nano-Se feeding) than Group 5 (control + day feed +day starvation) concerning other feeding regimes.

From the previously obtained result, our data revealed that the second feeding regime (Nano-Se + day feed + 1-day starvation) gives the best feeding regime, which reflects a marked improvement

TABLE 5 Effect of Nano-SE particles on the haematological and biochemical parameters of *Mugil cephalus* after 12 weeks of experiment

	G1	G2	G3	G4	G5	G6	*SE*	*p* value
RBCs	3.76^{a}	3.92^{a}	3.50^{b}	3.28^{c}	3.58^{b}	3.17^{c}	0.04	0.00
Hb	11.34^{b}	11.90^{a}	10.52^{c}	10.10^{d}	10.78^{c}	10.02^{d}	0.15	0.00
PCV	37.00^{b}	38.50^{a}	34.50^{c}	32.00^{d}	35.00^{c}	31.00^{e}	0.70	0.00
MCV	98.39	98.21	98.57	97.57	97.77	97.95	1.02	0.91
MCH	30.16	30.35	30.05	30.80	30.11	30.65	0.15	0.06
MCHC	30.66	30.90	30.48	31.56	30.80	31.31	0.29	0.06
WBCs	14.21	14.83	12.32	12.69	13.59	12.16	1.10	0.57
Heterophil	1.42	1.34	1.67	1.78	1.29	1.89	2.09	0.85
Lymphocyte	11.58	12.23	9.67	9.90	11.01	9.00	3.09	0.80
Monocyte	1.07^{b}	1.18^{a}	0.86^{c}	0.88^{c}	1.15^{c}	0.91^{ab}	0.95	0.02
Eosinophil	0.07	0.08	0.06	0.07	0.07	0.18	0.64	0.42
Basophil	0.07	0.00	0.06	0.07	0.07	0.18	0.57	0.70
Lysozyme	13.26^{b}	13.81^{a}	10.46^{c}	10.32^{c}	11.11^{c}	10.24^{c}	0.10	0.00
Phagocytic activity	13.05^{b}	14.15^{a}	11.49^{c}	11.34^{d}	11.58^{c}	11.26^{d}	0.09	0.00
Phagocytic index	1.29^{b}	1.38^{a}	1.07^{e}	1.16^{d}	1.29^{c}	1.03^{e}	0.06	0.01
Total protein	4.71^{b}	4.81^{a}	4.30^{c}	4.13^{d}	4.22^{d}	4.15^{d}	0.03	0.00
Albumin	1.73	1.72	1.78	1.75	1.72	1.80	0.03	0.30
Globulin	2.98^{b}	3.09^{a}	2.53^{c}	2.39^{d}	2.50^{c}	2.35^{d}	0.03	0.00
AST	18.14^{c}	18.11^{c}	20.00^{a}	19.81^{a}	18.49^{b}	20.04^{a}	0.21	0.00
ALT	21.62^{c}	21.82^{c}	22.98^{b}	23.00^{a}	21.25^{c}	23.09^{a}	0.64	0.09
MDA	18.15^{d}	17.63^{d}	22.37^{c}	24.09^{a}	20.28^{b}	24.39^{a}	0.29	0.00
GPx	17.17^{b}	17.45^{a}	16.24^{c}	15.30^{d}	16.69^{c}	14.35^{e}	0.22	0.00
CAT	11.32^{b}	11.67^{a}	10.54^{c}	10.33^{cd}	11.01^{c}	10.00^{d}	0.06	0.00
SOD	11.11^{b}	11.26^{a}	10.89^{c}	10.57^{c}	10.82^{c}	10.32^{d}	0.08	0.00

Note: Values are expressed as means ± *SE*. Different superscript letters indicate significant differences in the same column.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MDA, malondialdehyde; Nano-SE, nanoselenium; PCV, packed cell volume; RBCs, red blood cells; SOD, superoxide dismutase; WBCs, white blood cells.

in all measured parameters concerning growth, haematology immunity, antioxidant and gene expression analysis.

4 | DISCUSSION

Fish farming is one of the most important economies of the world, and one of the essential goals in fish research is to reduce production costs while preserving and improving the vital functions, weight and immunity of fish, so we conducted this study to investigate the Nano-Se particle in different feeding regime.

Our result revealed that the addition of Nano-Se particles to the diet of fish with feeding regime of feeding day followed by a day of starvation significantly showed significant improvement in haematological picture and leukocytes as shown in Tables 3 and 4 in *O. niloticus* and *M. cephalus* fish concerning other treated groups; this result was inconsistent with Neamat-Allah et al. (2019); they reported that selenium nanoparticles denote leukocytosis in Nile tilapia fish due to the protecting effect of Nano-Se that avoids the erythrocyte from hemolysis either by influential antioxidant impact (Qiang et al., 2017). Our result concerning growth efficiency revealed that the FBW, WG and specific growth weight of both *O. niloticus* and *M. cephalus* fish were significantly improved in Group 2 (Nano-Se + day feed + 1-day starvation), and these data usually agreed with earlier research that assesses the role on Nano-Se particles in various species of fish (Ashouri et al., 2015; Dawood, Koshio, Zaineldin, Van Doan, Ahmed, et al., 2019; Lin et al., 2010). The level of selenium needed to achieve optimum growth efficiency may vary based on the type of selenium, the time of administration and the experimental technique, as well as the fish and fish species (Lee et al., 2008).

Growth and feed efficiency improvements in Nano-Se treated groups are due to stimulating growth hormone development, selenoprotein synthesis, activation of intestinal protease enzymes and increased intracellular protein content (Khan et al., 2017). Besides, selenium acts as an aco-enzyme in the stimulation of protease and lipase (Shenkin, 2006) and improves the digestibility and use of proteins by increasing the number of intestinal microbes

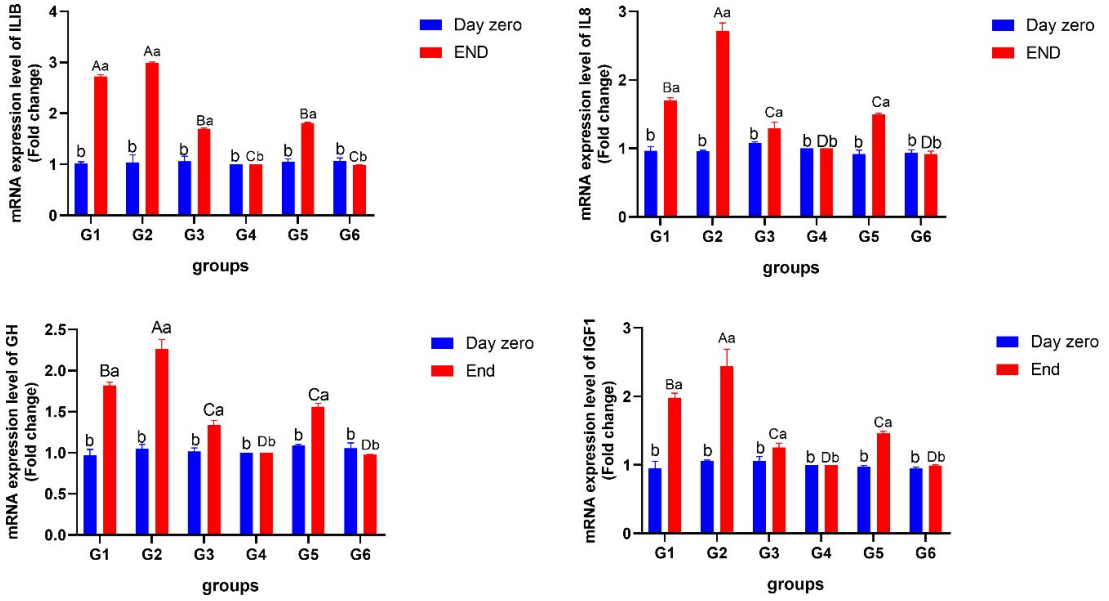

FIGURE 1 Effect of feeding Nano-Se particles on the mRNA expression genes on *O. niloticus* fish. Values are expressed as means ± standard error. Means within a column not sharing a common superscript significantly differ from each other. $P < 0.05$

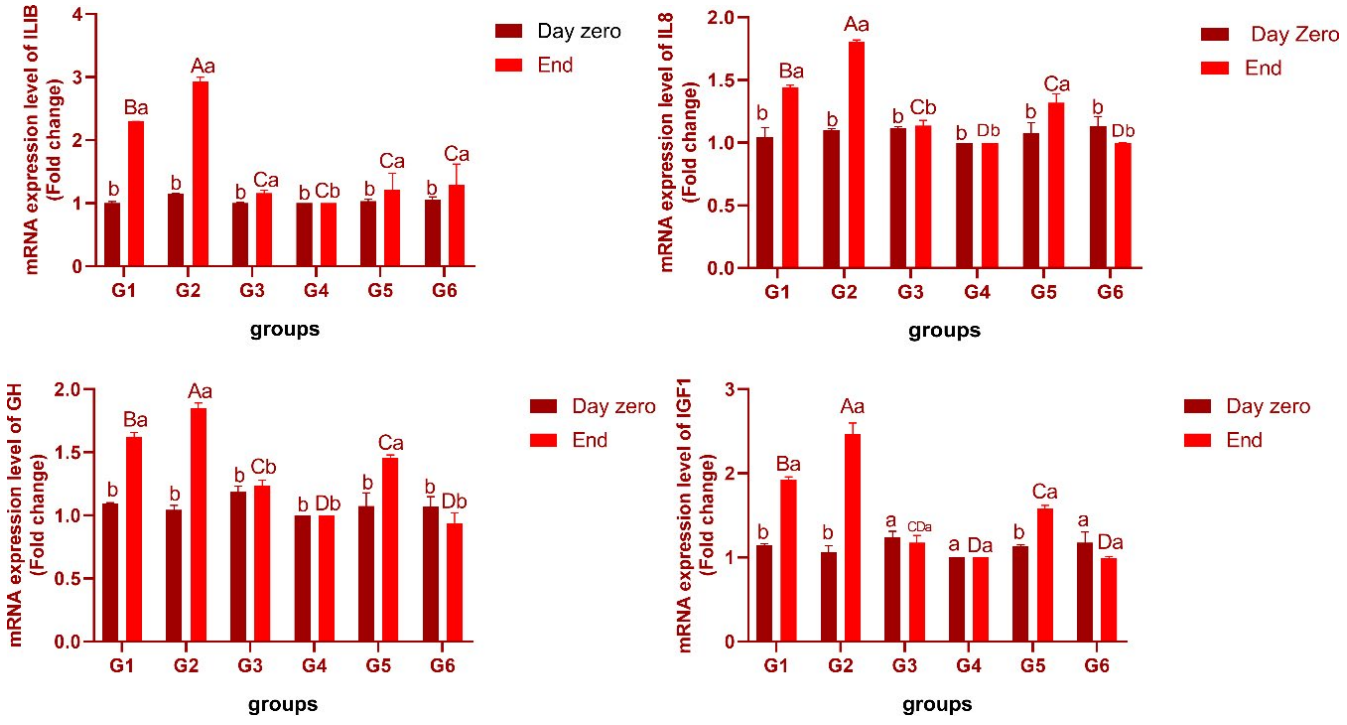

FIGURE 2 Effect of feeding Nano-Se particles on the mRNA expression genes on *M. cephalus* fish. Values are expressed as means ± standard error. Means within a column not sharing a common superscript significantly differ from each other. $P < 0.05$

number and operating digestive proteases (Chaudhary et al., 2010; Shi et al., 2011). Our result revealed that the (Nano-Se + day feed + 1-day starvation) showed a significant markedly increase in total protein in line with substantial improvement in lysosomal and phagocytic activity; this result was inconsistent with (Dawood et al., 2020). They focused on the importance of selenium as an immunostimulant through its role in activating lysosomal and phagocytic activity (Harikrishnan et al., 2011).

Our result revealed that Nano-Se treated group (Nano-Se + day feed + 1-day starvation) showed markedly decreased MDA with a consequent increase in GPx, CAT and superoxide activity; this result was in harmony with Dawood et al. (2020). Selenium acts as an

TABLE 6 Effect of Nano-SE particles on the growth weight parameters of *Oreochromis niloticus* after 12 weeks of experiment

	G1	G2	G3	G4	G5	G6	*p* value	*SE*
IW	25.4850	25.0650	25.3900	26.1250	24.7800	25.6350	0.4140	0.2100
FW	97.03^{b}	107.26^{a}	80.575^{d}	78.945^{e}	92.75^{c}	71.49^{f}	0.0000	3.5800
WG	71.545^{b}	82.195^{a}	55.18^{d}	52.82^{d}	67.97^{c}	45.855^{e}	0.0000	1.9400
DWG	0.85^{b}	0.98^{a}	0.655^{d}	0.625^{e}	0.81^{c}	0.545^{f}	0.0000	0.0200
SGR	1.595^{b}	1.73^{a}	1.375^{d}	1.32^{e}	1.57^{c}	1.22^{f}	0.0000	0.0500

Note: Values are expressed as means ± *SE*. Different superscript letters indicate significant differences in the same column.

Abbreviations: DWG, daily weight gain; FW, final body weight; IW, initial body weight; Nano-SE, nanoselenium; SGR, specific growth rate; WG, weight gain.

TABLE 7 Effect of Nano-SE particles on the growth weight parameters of *Mugil cephalus* after 12 weeks of experiment

	G1	G2	G3	G4	G5	G6	*p* value	*SE*
IW	30.92	30.07	30.515	31.005	30.91	30.68	0.0000	0.3
FW	100.41^{b}	105.16^{a}	88.48^{c}	82.535^{d}	93.705^{c}	80.67^{d}	0.0000	2.8000
WG	69.49^{b}	75.09^{a}	57.965^{d}	51.53^{e}	62.795^{c}	49.99^{e}	0.0000	1.9000
DWG	0.825^{b}	0.895^{a}	0.69^{d}	0.61^{e}	0.745^{c}	0.595^{e}	0.0000	0.02
SGR	1.41^{b}	1.46^{a}	1.4400	1.4500	1.3900	1.4300	0.0000	0.0100

Note: Values are expressed as means ± *SE*. Different superscript letters indicate significant differences in the same column.

Abbreviations: DWG, daily weight gain; FW, final body weight; IW, initial body weight; Nano-SE, nanoselenium; SGR, specific growth rate; WG, weight gain.

antioxidant in that it forms "selenocysteine," a component of GPX 's active core (Terova et al., 2018) and to the antioxidant activity of selenium (Saffari et al., 2017). SOD, CAT and GPX activities as essential antioxidant enzymes can be considered markers of oxidative injury (Dawood, Koshio, Zaineldin, Van Doan, Moustafa, et al., 2019). Nevertheless, MDA is a highly toxic material formed by the decomposition of lipid peroxides, which can cause hurt to the body (Yao et al., 2010). The increase in antioxidant parameters in fish after Nano-Se utilization may be due to Se's involvement in the formation of selenocysteine in the active centre of the GPX enzyme (Köhrle et al., 2000).

Our result revealed the upregulation of mRNA expression of GH and IGF-1 in the Nano-Se treated group, especially Groups 2 (Nano-Se + day feed + 1-day starvation) and 1 (continuous feeding of Nano-Se) concerning other groups as shown in Figures 1 and 2. This result was in line with Cupaioli et al. (2014) in which they confirmed that selenium enhanced the growth hormone. Consequently, the obtained result concerning growth hormone and IGF-1 expression supported our growth efficiency results. Abarike et al. (2019) reported that Nano-Se upregulates the pro-inflammatory cytokines, which help our finding in Figures 1 and 2 that showed the marked upregulation of IL1B and IL8 in *O. niloticus* and *M. cephalus* fish that previously treated with Nano-Se particle. IL-1β is a pro-inflammatory cytokine that stimulates the lymphocytes and macrophages against disease (Low et al., 2003). Restricted feeding schemes may be practical tools to boost fish output efficiency (Kumar et al., 2017). Our result revealed that 1-day feeding followed by 1-day starvation improves all physiological parameters and growth rate efficiency. Two potential causes for the offsetting growth of hyperphagia or a combination of hyperphagia and improved feed quality were reported by (Ye et al., 2016). Our data concerning the immunostimulant activity of Nano-Se was also supported by Dawood, Zommara, et al., (2019) in which they found that Nano-Se upregulates pro-inflammatory cytokines, especially IL-1β.

5 | CONCLUSION

Nano-Se supplementation with a dietary regime feeding the fish 1 day followed by 1-day starvation and soon tends to potentiate the growth efficiency and immunity and improve the growth hormone, insulin growth factors and pro-inflammatory cytokines. For the first time, these findings supported our hypothesis that confirms the Nano-Se supplementation to the diet of fish with special dietary regime could be useful for aquatic life and economy in decreasing the feeding cost and increasing the fish health welfare and growth.

ACKNOWLEDGEMENT

The authors extend their appreciation to Taif University for funding current work by Taif University Researchers Supporting Project number (TURSP-2020/119), Taif University, Taif, Saudi Arabia.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Eman M. Moustafa: Investigation; Visualization; Writing-original draft; Writing-review & editing. **Foad Farrag:** Software. **Montaser M. Hassan:** Resources; Supervision; Visualization. **Amira Omar:** Formal analysis. **Ahmed G. Gewida:** Formal analysis; Funding acquisition; Validation; Visualization. **Mohammed F. Abd-Elghany:** Visualization; Writing-original draft.

ETHICAL APPROVAL

The study's experimental architecture and procedures won approval from the clinical treatment and use of animals Kafrelsheikh University Board, Kafrelsheikh, Egypt.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.490.

DATA AVAILABILITY STATEMENT

Data were available upon a request from the corresponding author.

ORCID

Eman M. Moustafa https://orcid.org/0000-0002-7788-8533
Montaser M. Hassan https://orcid.org/0000-0002-7990-6969
Mustafa Shukry https://orcid.org/0000-0003-2722-2466

REFERENCES

Abarike, E. D., Kuebutornye, F. K., Jian, J., Tang, J., Lu, Y., & Cai, J. (2019). Influences of immunostimulants on phagocytes in cultured fish: A mini review. *Review in Aquaculture*, *11*, 1219–1227. https://doi.org/10.1111/raq.12288

Abd El-Kader, M. F., Fath El-Bab, A. F., Abd-Elghany, M. F., Abdel-Warith, A.-W.-A., Younis, E. M., & Dawood, M. A. O. (2020). Selenium nanoparticles act potentially on the growth performance, hemato-biochemical indices, antioxidative, and immune-related genes of european seabass (*Dicentrarchus labrax*). *Biological Trace Element Research*. https://doi.org/10.1007/s12011-020-02431-1

Abdel-Mageid, A. D., Zaki, A. G., El Senosi, Y. A., Fahmy, H. A., El Asely, A. M., Abo-Al-Ela, H. G., & El-Kassas, S. J. A. R. (2020). Modulatory effect of lipopolysaccharide on immune-related gene expression and serum protein fractionation in grey mullet, *Mugil cephalus*. *Aquaculture Research*, *51*, 1643–1652.

Ashouri, S., Keyvanshokooh, S., Salati, A. P., Johari, S. A., & Pasha-Zanoosi, H. J. A. (2015). Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*). *Aquaculture*, *446*, 25–29. https://doi.org/10.1016/j.aquaculture.2015.04.021

Blaxhall, P., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*(6), 771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Byamungu, N., Darras, V., & Kühn, E. J. A. (2001). Growth of heat-shock induced triploids of blue tilapia, *Oreochromis aureus*, reared in tanks and in ponds in Eastern Congo: Feeding regimes and compensatory growth response of triploid females. *Aquaculture*, *198*, 109–122. https://doi.org/10.1016/S0044-8486(00)00605-0

Chan, C.-R., Lee, D.-N., Cheng, Y.-H., Hsieh, D.-J.-Y., & Weng, C.-F. (2008). Feed deprivation and re-feeding on alterations of proteases in tilapia *Oreochromis mossambicus*. *Zoological Studies-Taipei*, *47*, 207.

Chaudhary, M., Garg, A. K., Mittal, G. K., & Mudgal, V. (2010). Effect of organic selenium supplementation on growth, Se uptake, and nutrient utilization in guinea pigs. *Biological Trace Element*, *133*, 217–226. https://doi.org/10.1007/s12011-009-8420-z

Crosetti, D. (2015). Current state of grey mullet fisheries and culture. In D. Crosetti & S. J. Blaber (Eds.), *Biology, ecology and culture of grey mullets (Mugilidae)* (pp. 398-450). CRC Press.

Cupaioli, F. A., Zucca, F. A., Boraschi, D., & Zecca, L. (2014). Engineered nanoparticles. How brain friendly is this new guest? *Progress in Neurobiology*, *119*, 20–38. https://doi.org/10.1016/j.pneurobio.2014.05.002

Daudpota, A. M., Abbas, G., Kalhoro, I. B., Shah, S. S. A., Kalhoro, H., Hafeez-ur-Rehman, M., & Ghaffar, A. J. (2016). Effect of feeding frequency on growth performance, feed utilization and body composition of juvenile Nile tilapia, *Oreochromis niloticus* (L.) reared in low salinity water. *Pakistan Journal of Zoology*, *48*, 171–177.

Dawood, M. A. O., Koshio, S., Zaineldin, A. I., Van Doan, H., Ahmed, H. A., Elsabagh, M., & Abdel-Daim, M. M. (2019). An evaluation of dietary selenium nanoparticles for red sea bream (*Pagrus major*) aquaculture: Growth, tissue bioaccumulation, and antioxidative responses. *Environmental Science and Pollution Research*, *26*(30), 30876–30884. https://doi.org/10.1007/s11356-019-06223-6

Dawood, M. A. O., Koshio, S., Zaineldin, A. I., Van Doan, H., Moustafa, E. M., Abdel-Daim, M. M., Angeles Esteban, M., & Hassaan, M. S. (2019). Dietary supplementation of selenium nanoparticles modulated systemic and mucosal immune status and stress resistance of red sea bream (*Pagrus major*). *Fish Physiology and Biochemistry*, *45*, 219–230. https://doi.org/10.1007/s10695-018-0556-3

Dawood, M. A., Zommara, M., Eweedah, N. M., & Helal, A. I. (2019). Synergistic effects of selenium nanoparticles and vitamin e on growth, immune-related gene expression, and regulation of antioxidant status of Nile tilapia (*Oreochromis niloticus*). *Biological Trace Element Research*, *195*(2), 624–635. https://doi.org/10.1007/s12011-019-01857-6

Dawood, M. A., Zommara, M., Eweedah, N. M., & Helal, A. I. (2020). The evaluation of growth performance, blood health, oxidative status and immune-related gene expression in Nile tilapia (*Oreochromis niloticus*) fed dietary nanoselenium spheres produced by lactic acid bacteria. *Aquaculture*, *515*, 734571. https://doi.org/10.1016/j.aquaculture.2019.734571

El Basuini, M., El-Hais, A., Dawood, M., Abou-Zeid, A. S., El-Damrawy, S., Khalafalla, M. S., Koshio, S., Ishikawa, M., & Dossou, S. J. A. N. (2017). Effects of dietary copper nanoparticles and vitamin C supplementations on growth performance, immune response and stress resistance of red sea bream, *Pagrus major*. *Aquaculture Nutrition*, *23*, 1329–1340.

Garcia, J. A., & Villarroel, M. (2009). Effect of feed type and feeding frequency on macrophage functions in tilapia (*Oreochromis niloticus* L.). *Fish & Shellfish Immunology*, *27*, 325–329. https://doi.org/10.1016/j.fsi.2009.05.018

Garcia, J., Villarroel, M. J. F., & Immunology, S. (2009). Effect of feed type and feeding frequency on macrophage functions in tilapia (*Oreochromis niloticus* L.). *Fish & Shellfish Immunology*, *27*(2), 325–329. https://doi.org/10.1016/j.fsi.2009.05.018

Harikrishnan, R., Kim, J.-S., Kim, M.-C., Balasundaram, C., & Heo, M.-S.-J.-A. (2011). *Prunella vulgaris* enhances the non-specific immune response and disease resistance of *Paralichthys olivaceus* against *Uronema marinum*. *Aquaculture*, *318*, 61–66. https://doi.org/10.1016/j.aquaculture.2011.05.020

Houston, A. J. (1990). Blood and circulation. In C. B. Schreck & P. B. Moyle (Eds.), *Methods for fish biology* (pp. 415–488). American Fisheries Society.

Huang, S., Wang, L., Liu, L., Hou, Y., & Li, L. (2015). Nanotechnology in agriculture, livestock, and aquaculture in China. A review. *Agronomy for Sustainable Development*, *35*, 369–400. https://doi.org/10.1007/s13593-014-0274-x

Jain, N. C. (1986). *Schalm's veterinary hematology*. Lea & Febiger.

Jobling, M. (2012). National Research Council (NRC): Nutrient requirements of fish and shrimp. *Aquaculture International*, *20*, 601–602. https://doi.org/10.1007/s10499-011-9480-6

Kawahara, E., Ueda, T., & Nomura, S. J. F. P. (1991). In vitro phagocytic activity of white-spotted char blood cells after injection with *Aeromonas salmonicida* extracellular products. *Fish Pathology*, *26*(4), 213–214. https://doi.org/10.3147/jsfp.26.213

Khan, K. U., Zuberi, A., Nazir, S., Ullah, I., Jamil, Z., & Sarwar, H. J. A. R. (2017). Synergistic effects of dietary nano selenium and vitamin C on growth, feeding, and physiological parameters of mahseer fish (*Tor putitora*). *Aquaculture Reports*, *5*, 70–75. https://doi.org/10.1016/j.aqrep.2017.01.002

Köhrle, J., Brigelius-Flohé, R., Böck, A., Gärtner, R., Meyer, O., & Flohé, L. (2000). Selenium in biology: Facts and medical perspectives. *Biological Chemistry*, *381*(9–10), 849–864. https://doi.org/10.1515/BC.2000.107

Kumar, P., Jain, K. K., MunilKumar, S., & Sudhagar, S. A. J. A. R. (2017). Alternate feeding strategies for optimum nutrient utilization and reducing feed cost for semi-intensive practices in aquaculture system—A review. *Agricultural Reviews*, *38*(2), 145–151. https://doi.org/10.18805/ag.v38i02.7946

Lee, S.-M., Hwang, U.-G., & Cho, S. H. (2000). Effects of feeding frequency and dietary moisture content on growth, body composition and gastric evacuation of juvenile Korean rockfish (*Sebastes schlegeli*). *Aquaculture*, *187*, 399–409. https://doi.org/10.1016/S0044-8486(00)00318-5

Lee, S., Lee, J.-H., & Bai, S. C. (2008). A preliminary study on effects of different dietary selenium (Se) levels on growth performance and toxicity in juvenile black seabream, *Acathopagrus schlegeli* (Bleeker). *Asian-Australasian Journal of Animal Sciences*, *21*(12), 1794–1799. https://doi.org/10.5713/ajas.2008.80285

Lin, Y.-H., Shih, C.-C., Kent, M., & Shiau, S.-Y.-J.-A. (2010). Dietary copper requirement reevaluation for juvenile grouper, *Epinephelus malabaricus*, with an organic copper source. *Aquaculture*, *310*(1–2), 173–177. https://doi.org/10.1016/j.aquaculture.2010.10.004

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*, 402–408. https://doi.org/10.1006/meth.2001.1262

Low, C., Wadsworth, S., Burrells, C., & Secombes, C. J. A. (2003). Expression of immune genes in turbot (*Scophthalmus maximus*) fed a nucleotide-supplemented diet. *Aquaculture*, *221*, 23–40. https://doi.org/10.1016/S0044-8486(03)00022-X

Lucky, Z., & Lucký, Z. (1977). *Methods for the diagnosis of fish diseases*. AGRIS Amerind Pub.

Naderi, M., Keyvanshokooh, S., Ghaedi, A., & Salati, A. P. (2019). Interactive effects of dietary Nano selenium and vitamin E on growth, haematology, innate immune responses, antioxidant status and muscle composition of rainbow trout under high rearing density. *Aquaculture Nutrition*, *25*(5), 1156–1168. https://doi.org/10.1111/anu.12931

Neamat-Allah, A. N., Mahmoud, E. A., Abd El Hakim, Y. (2019). Efficacy of dietary Nano-selenium on growth, immune response, antioxidant, transcriptomic profile and resistance of Nile tilapia, *Oreochromis niloticus* against *Streptococcus iniae* infection. *Fish & Shellfish Immunology*, *94*, 280–287. https://doi.org/10.1016/j.fsi.2019.09.019

Pacitti, D., Lawan, M., Feldmann, J., Sweetman, J., Wang, T., Martin, S., & Secombes, C. J. (2016). Impact of selenium supplementation on fish antiviral responses: A whole transcriptomic analysis in rainbow trout (*Oncorhynchus mykiss*) fed supranutritional levels of Sel-Plex®. *BMC Genomics*, *17*(1). https://doi.org/10.1186/s12864-016-2418-7

Pang, J.-C., Gao, F.-Y., Lu, M.-X., Ye, X., Zhu, H.-P., & Ke, X.-L. (2013). Major histocompatibility complex class IIA and IIB genes of Nile tilapia *Oreochromis niloticus*: Genomic structure, molecular polymorphism and expression patterns. *Fish & Shellfish Immunology*, *34*, 486–496. https://doi.org/10.1016/j.fsi.2012.11.048

Parry Jr., R. M., Chandan, R. C., & Shahani, K. M. (1965). A rapid and sensitive assay of muramidase. *Biology and Medicine*, *119*, 384–386. https://doi.org/10.3181/00379727-119-30188

Pouomogne, V., & Ombredane, D. J. T. (2001). Effect of feeding frequency on the growth of tilapia (*Oreochromis niloticus*) in earthen ponds. *Tropicultura*, *19*, 141–146.

Qiang, J., Tao, F., He, J., Sun, L., Xu, P., & Bao, W. (2017). Effects of exposure to *Streptococcus iniae* on microRNA expression in the head kidney of genetically improved farmed tilapia (*Oreochromis niloticus*). *BMC Genomics*, *18*(1), https://doi.org/10.1186/s12864-017-3591-z

Qiang, J., Wang, H., Li, R.-W., & Peng, J. J. (2009). Effects of feeding frequency on growth, body biochemical composition and digestive enzymes of larvae and juvenile of hybrid tilapia (*Oreochromis niloticus*× *O. aureus*). *Journal of Guangdong Ocean University*, *4*.

Riche, M., Haley, D., Oetker, M., Garbrecht, S., & Garling, D. J. A. (2004). Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.). *Aquaculture*, *234*(1-4), 657–673. https://doi.org/10.1016/j.aquaculture.2003.12.012

Riche, M., Oetker, M., Haley, D. I., Smith, T., & Garling, D. L. (2004). Effect of feeding frequency on consumption, growth, and efficiency in juvenile tilapia (*Oreochromis niloticus*). *The Israeli Journal of Aquaculture—Bamidgeh*, *56*(4), 247–255.

Saffari, S., Keyvanshokooh, S., Zakeri, M., Johari, S., & Pasha-Zanoosi, H. J. A. N. (2017). Effects of different dietary selenium sources (sodium selenite, selenomethionine and nanoselenium) on growth performance, muscle composition, blood enzymes and antioxidant status of common carp (*Cyprinus carpio*). *Aquaculture*, *23*, 611–617.

Saffari, S., Keyvanshokooh, S., Zakeri, M., Johari, S. A., Pasha-Zanoosi, H., & Mozanzadeh, M. T. (2018). Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry*, *44*(4), 1087–1097. https://doi.org/10.1007/s10695-018-0496-y

Shenkin, A. (2006). Micronutrients in health and disease. *Postgraduate Medical Journal*, *82*(971), 559–567. https://doi.org/10.1136/pgmj.2006.047670

Shi, L., Xun, W., Yue, W., Zhang, C., Ren, Y., Liu, Q., Wang, Q., & Shi, L. (2011). Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. *Animal Feed Science and Technology*, *163*(2-4), 136–142. https://doi.org/10.1016/j.anifeedsci.2010.10.016

Suttle, N. F. (2010). *Mineral nutrition of livestock*. CABI.

Terova, G., Rimoldi, S., Izquierdo, M., Pirrone, C., Ghrab, W., & Bernardini, G. (2018). Nano-delivery of trace minerals for marine fish larvae: influence on skeletal ossification, and the expression of genes involved in intestinal transport of minerals, osteoblast differentiation, and oxidative stress response. *Fish Physiology and Biochemistry*, *44*(5), 1375–1391. https://doi.org/10.1007/s10695-018-0528-7

Thongprajukaew, K., Kovitvadhi, S., Kovitvadhi, U., & Preprame, P. (2017). Effects of feeding frequency on growth performance and digestive enzyme activity of sex-reversed Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). *Agriculture and Natural Resources*, *51*, 292–298. https://doi.org/10.1016/j.anres.2017.04.005

Tung, P.-H., & Shiau, S.-Y. (1991). Effects of meal frequency on growth performance of hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, fed different carbohydrate diets. *Aquaculture*, *92*, 343–350. https://doi.org/10.1016/0044-8486(91)90039-A

Wassef, E., El Masry, M., & Mikhail, F. J. A. R. (2001). Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. *Aquaculture Research 32*, 315–322.

Yao, J., Wang, J.-Y., Liu, L., Li, Y.-X., Xun, A.-Y., Zeng, W.-S., Jia, C.-H., Wei, X.-X., Feng, J.-L., Zhao, L. I., & Wang, L.-S. (2010). Anti-oxidant effects of resveratrol on mice with DSS-induced ulcerative colitis. *Archives of Medical Research*, *41*(4), 288–294. https://doi.org/10.1016/j.arcmed.2010.05.002

Ye, J. D., Chen, J. C., & Wang, K. J. (2016). Growth performance and body composition in response to dietary protein and lipid levels in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) subjected to normal and temporally restricted feeding regimes. *Journal of Applied Ichthyology*, *32*, 332–338.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Moustafa EM, Abd El-Kader MF, Hassan MM, et al. Trial for use nanoselenium particle with different dietary regime in *Oreochromis niloticus* and *Mugil cephalus* polyculture ponds: Growth efficiency, haematological, antioxidant, immunity and transcriptional analysis. *Vet Med Sci*. 2021;7:1575–1586. https://doi.org/10.1002/vms3.490