Fish and Shellfish Immunology 94 (2019) 280–287

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Efficacy of dietary Nano-selenium on growth, immune response, antioxidant, transcriptomic profile and resistance of Nile tilapia, *Oreochromis niloticus* against *Streptococcus iniae* infection

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ARTICLE INFO

Keywords:
Immunoglobulin M
Immune response
Genes expression
Growth
Oxidant
Oreochromis niloticus
Streptococcus iniae
Nano-Se
Sodium selenite

ABSTRACT

As recently applicable, there are few studies on the impact of using nano-selenium (nano-Se) on varied fish species. Where nothing reachable focused on its impact on tilapias so, the present analysis evaluated the efficacy of using nano-Se in tilapias on immune response, antioxidant defense compared by conventional Se form. 480 *O. niloticus* fingerlings were haphazardly grouped firstly into three groups with four replicates of each. The control one (CT) was fed on a basal diet. The second and third one supplemented with 0.7 mg/kg^{-1} Se and nano-Se respectively for ten weeks. At the start day of the ninth week, two replicates from each group were injected by *Streptococcus iniae* where, the remaining replicates stand without challenge. Enhancement of growth performance measurements were noted in nano-Se compared to Se or CT groups. Existed anemia in *S. iniae* tilapias became alleviated by using nano-Se that also, improves the alteration of leucogram induced by challenge. Elevation of aminotransferases, alkaline phosphatase, lactate dehydrogenase (ALT, AST, ALP and LDH) and creatinine in Se and CT challenged replicates that seemed nearly normal by using nano-Se. Usage of nano-Se showed more powerful antioxidant activities than Se. There were an expansion of immunoglobulin M, lysozymes, glutathione peroxidase, nitric oxide, superoxide dismutase and catalase (IgM, LYZ, GPx, NO, SOD, CAT) and their related gene expression in nano-Se with contrast in Se or CT challenged groups. Nile tilapias challenged by *S. iniae* disclosed substantial expansion in the percentage of mortality in CT challenged fish (93.33%), followed by the group supplemented with Se (73.33%), whereas the lowermost one at fish supplemented by nano-Se (26.66%). The mortalities have been stopped from the 5th, 12th and 14th days in, nano-Se, Se and CT respectively. It can be concluded that using of Se 0.7 mg/kg^{-1}induce immunosuppressive, antioxidant, liver and kidneys negative impact on tilapias where the same dose from nano-Se was more potent immunomodulating and antioxidant. Also it is attend in counteracting the serious impact induced by *S. iniae* challenge.

1. Introduction

Dietary minerals are commonly trace elements. These are fundamental in many of metabolism in living organism. Selenium (Se) is one of these parts. It performs a vital roles in antioxidant resistance techniques, adjustment of thyroid hormone metabolism, and cell growth [1]. It participates in selenoproteins compounds as glutathione peroxidase (GPx) and affords shelter against oxidative impairment [2].

Despite adequate Se consumption is required, it possess a narrow therapeutic index [3,4]. In fish, the susceptibility to infection, growth reduction, and immune suppression are an early consequences of selenium deprivation. The optimal Se necessities fluctuated from 0.15 to 0.7 mg kg^{-1} in diverse fish species [5]. Accumulation of Se more than needs requirements might cause harm to organisms [6,7].

Nowadays, nanotechnology is the growing topic that has varied purpose in the field of aquaculture. Nanoparticles reveal elevated potential than their conventional form, as they possess extreme surface area, which quickly interact with other organic molecules. Thus, diets supplemented with nanoparticles augments the gut absorption capability [8]. Recently, using of nano-Se in fish diets has bioavailability, low toxicity in addition to improve the immune reaction and antioxidant defense in common carp [9,10] and rainbow trout [11].

Nile tilapia, is a prevalent fish class cultured primarily in Egypt, ascribed to its virtuous expansion and extreme marketing profit [12]. Fish pathogens are largely controlled using antibiotics. But, constant severe usage of them may lead to the progress of unfavorable drug

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https://doi.org/10.1016/j.fsi.2019.09.019
Received 3 August 2019; Received in revised form 3 September 2019; Accepted 5 September 2019
Available online 06 September 2019

resistance that diminished the efficiency of drugs [13]. Streptococcosis is a global serious trouble for fish production. It is combined with high commercial losses. Now, *Streptococcus iniae* have been pronounced as pathogenic to fish. Outbreaks from streptococcosis were found in North America, Middle East, and Asia Pacific [14]. Sometimes it causes 100% fatality in tilapias after 14 day post intraperitoneal challenge. It resist most of commercial antibiotics [15].

There is no obtainable documents around the influence of using nano-Se in Nile tilapia on immunity, antioxidant defense and resistance against *S. iniae*, where this work pointed to assess these parameters in tilapias after supplementation with conventional and nano-Se.

2. Materials and methods

2.1. Nile tilapia

A sum of 480 tilapias fingerlings were acquired from the Abbassa Fish Hatchery in EL- Sharqia Region, Egypt with an average body weight of 33 ± 0.29 g. Tilapias were habitual for fifteen days earlier the start of the research in glass aquaria and all requirements.

2.2. Tested compounds

Inorganic Se (sodium selenite), Na_2SeO_3, 99. % purity and nano-Se with 99.95% purity and 30–45 nm particle size were gained from Sigma-Aldrich and Nano Technology Center at Faculty of pharmacy, Al-Azhar University separately.

2.3. Experimental design

Fish were haphazardly grouped firstly into three groups, and each group retained four replicates in glass aquaria. The control group (CT) was fed on a basal diet only without any Se supplements [16] (Table 1). The diets were made at the Fish Research Unit, at Veterinary Medicine, Zagazig University in Egypt. It had 2944.42 kcal/kg metabolizable energy and 30.83% CP in the shape of pellets and was prepared to congregate the nutrient essentials of *O. niloticus* [5,17]. Se group was fed on the basal diet accompanied with 0.7 mg/kg^{-1} sodium selenite where the third group was supplemented with nano-Se with the same dose [10]. Tilapias were maintained with experimental diets for 10 weeks afterward; where at the start day of the ninth week, two replicates from each group were injected by *S. iniae* where, the remaining replicates stand without infection. All tilapias in the varied groups were weighed at the beginning and at the end of 9th week for estimation growth performance measurements as: weight gain (WG) = final - initial weight, and feed conversion ratio (FCR) = given food (weight) / total weight gain. Corresponding to Ethics of Animal Usage in Research Committee (EAURC) at Zagazig University. The experimental manages has indorsed which were ensued the NIH general guidelines for Care and Use of Laboratory Animals in scientific investigations.

Table 1
Ingredients and calculated composition of the experimental diet (% on dry matter basis).

Kg / Ingredient		%	Calculated composition
35	Yellow corn	84.28	Dry matter (DM)
10	Wheat flour	30.79	Crude protein (CP)
18	Soybean meal	9.92	Ether extract, (EE)
16	Fish meal	2.40	Crude fiber, (CF)
14	Poultry byproduct meal	7.09	Ash
5.5	Vegetable oil	38.99	Nitrogen-free extract, (NFE)
1.5	Se free premix[a]	2945.43	Digestible energy, (DE, kcal/kg diet)

[a] Se free premix: each 1 kg had 8600 IU vit. D3, 580000 IU vit. A, 142 mg vit. K3, 0.1 mg vit. C, 720 mg vit. E., 34 mg vit. B2, 58 mg vit. B1, 58 mg vit. B12, 86 mg folic acid, 34 mg vit. B6, 8 mg pantothenic acid, 2000 mg iron sulfate, 3000 mg zinc methionine, 65 mg manganese sulfate, 25 mg calcium iodide, 3400 mg copper sulfate, 572 mg cobalt sulfate, and calcium carbonate (hauler ingredient) to 1000 g. The protein levels of fish meal and soymeal was 65.2 and 43.4 respectively. They obtained from EL-Sharkia Company for animal nutrient, Egypt.

2.4. Challenge test

Afterward, at the start day of the ninth week of the experimental period, two replicates from each group were intraperitoneally injected with 25 μl of pathogenic *S. iniae at* concentration (1.2×10^8 CFU/ml) [13] of that former secluded from sickly fish and predictable by the automated system for bacterial classification (VITEK®, Bio-Merieux Inc., France) consequential to the companionship strategies. The pathogenicity test for settling LD_{50} was completed as portrayed [18]. The remaining two replicates stand without challenge. Thereafter injection, fifteen fish were held beneath observation for two weeks of challenged replicates for cumulative mortalities, where the other tilapias from all replicates were used for testing the varied parameters in this report.

2.5. Sampling

2.5.1. Blood sampling

After last day of the nineth weeks, the blood samples from all replicates were gained by needle perforating the caudal vein then extracted with potassium salt of EDTA [19–22] anticoagulant or without for hematological consideration and serum disjunction by centrifuge at 3000 rpm / 20 min [23,24] for the consideration of some immunological and biochemical markers.

2.5.2. Tissue sampling

Spleen and liver specimens were speedily stored at −80 °C after cervical amputation for real-time PCR and antioxidants enzymatic assay individually.

2.6. Hemogram analysis

The total count of leucocytes and erythrocytes (TLCs and RBCs) were completed conducting by Neubauer haemocytometer using Natt and Herrick diluting fluid. Giemsa's blood smear were completed for relative differential of leucocytes [25].

2.7. Immune response and biochemical profile markers

Nitric oxide (NO) [26] concisely, 85 μl (serum) were deproteinized with 30% zinc sulfate and reduction of nitrate to nitrite by vanadium chloride. Total nitrite, an indicator of NO was established at 540 nm. Lysozymes [27] In concise, 0.03% of *M. lysodeikticus* was dispersed in 0.05 mM (sodium phosphate buffer) exploited as a substrate. Ten microliter of homogenate was reared with 250 μl substrate /37 °C and then measured at 490 nm. IgM [28] utilized by an strip plate ELISA (enzyme-linked immunosorbent assay) kit for investigating the occurrence of IgM. Serum protein electrophoresis was quantified corresponding to a method that was beforehand recognized [29]. ALP, in concise, measured at 405 nm after mixing of 150 μl (10 mM ammonium bicarbonate) containing 1 mM $MgCl_2$ for 15 min at 37 °C, then evolution, 50 μl (4 mM p-nitrophenol phosphate). Aminotransferases, LDH and creatinine were estimated [30–33] correspondingly by supplied kits from Diamond Diagnostics Egypt.

2.8. Antioxidant biomarkers

Liver activities of GPx (glutathione peroxidase) [34] GPx vitiates H_2O_2 in the occurrence of GSH and expressed by mg/protein. SOD (superoxide dismutase) [35] concisely, admixture of 1.2 ml of sodium pyrophosphate buffer, 100 μl (phenazine methosulfate), 300 μl (nitro-blue tetrazolium), 200 μl (homogenate) and 2.8 ml (water) then add

Table 2
Primer sequences.

Reference	Primer Sequence (5'→3')	Gene name
LYZ	F:5′AGCCGTCCTGAAGAATGGAT3' F:5′GCATTGCTCTTGTGTCCACTT3'	[41]
IgM	F:5′AGGAGACAGGACTGGAATGCACAA3′ F:5′GGAGGCAGTATAGGTATCATCCTC3′	[38]
SOD	F:5′GGCTTTGATAAGGACAGTGGAAGACT3′ R:5′GAAGTGGGACGAGACCTGTAGTG3′	[39]
GPx	F:5′CCAAGAGAACTGCAAGAACGA3′ R:5′CAGGACACGTCATTCCTACAC3′	[39]
β-actin	R:5′CAG CAA GCA GGA GTA CGA TGAG3′ R:5′TGT GTG GTG TGT GGT TGT TTTG3′	[40]

LYZ = Lysozymes, IgM = Immunoglobulin M; SOD = superoxide dismutase; GPx = Glutathione peroxidase.

200 μl of NADH and considered at 560 nm. CAT (catalase) [36] concisely, using 13.2 mM (H_2O_2) in 50 mM (phosphate buffer with pH 7.0) and 100 μl of homogenate then considered at 240 nm. MDA (malondialdehyde) [37] concisely, 200 μl (homogenate) with 2 ml (thiobarbituric acid TBA) reagent including 0.375% TBA, 15% (trichloroacetic acid), and 0.25 (NHCl), then boiled/15 min, cooled and centrifuged and considered at 532 nm.

2.9. Analysis of immune-related and anti-oxidant genes

Quantitative real-time polymerase chain reaction (RT-qPCR) was operated to establish the equivocations in the expression intensities of spleen LYZ and IgM where, liver SOD and GPx in tilapias by gene-specific primer pairs were done according to Refs. [38–41] respectively as in (Table 2). The total RNA was liberated from tissue by the RNeasy Kit (Qiagen, Germany) consequent to the company's strategies. The intensity of RNA was tested by spectrophotometer ruminating the 260/280 nm ratio and the RNA integrity was gauged by electrophoresis. The total RNA was reverse derivative exploiting a QuantiTect Reverse Transcript kit (Qiagen, Germany) consequent to the company's strategies. The qPCR of the elected genes and housekeeping gene completed by a Rotor-Gene Q cycler (Qiagen, Germany) and QuantiTect SYBR Green PCR kits (Qiagen, Germany).

2.10. Statistical analysis

By two way analysis of variance (ANOVA) exploiting the SPSS 20.0 computer program. Duncan's multiple diversity test was run to compete variances means, and the significance was termed as $p < 0.01$. All data were computed as mean ± SE [42].

3. Results

3.1. Effect of varied forms of Se on growth performance of Nile tilapia

The detailed growth performance measurements (Table 3) such as final body weight (FBW), BWG % and SGR (%) were higher in nano-Se group than in the CT. FCR of tilapias that fed the diet containing nano-Se was significantly lower than that of those that fed Se and a basal diets.

3.2. Effect of varied forms of Se on hematology of Nile tilapia pre- and post-challenge with S. iniae infection

Concerning to erythrogram profile as publicized in Table 4 refers to non-significant deviations in erythrocytic count, hemoglobin level or hematocrit percentage neither their related blood indices (MCV, MCH & MCHC) in non-challenged replicates of Se or nano-Se in matching with the normal control. Whereas the challenged replicates reveals significant decline in RBCs number and Hb level in control and Se groups while the nano-Se group remain unchanged. Moreover the Ht % appeared dwindled in all of the three challenged groups comparing to the normal non-challenged control. An obvious rise in MCV was detailed in CT and Se challenged replicates without any fluctuation in MCH in any of experimental groups. Decline of MCHC was extant at the challenged CT group.

According to leucogram analysis as visualized in Table 4 nano-Se denotes to leukocytosis in non-challenged replicates compared to the normal control. This leukocytosis was more noticeable in the all of the challenged replicates compared to the control one. Lymphocytosis was clear in Se non-challenged replicate, while the nano-Se groups performed lymphocytosis only in the non-challenged replicate where it was closely normal in the challenged replicate. Heterophilia was pictured in all of challenged three replicates comparing to normal while the heterophils number in non-challenged groups remain untouched. Monocytosis was described in selenium and nano-Se groups either challenged or none while the proliferation was enormous in the challenged ones. Eosinophil passed without any variations in all of experimental groups rivaled to normal control. Existence of basophilia in challenged control replicate.

3.3. Effect of varied form of Se on biochemical parameters of Nile tilapia pre- and post-challenge with S. iniae infection

Screening of proteinogram (Table 5) concluded hyerproteinemia and hyperglobulinemia in non-challenged Se or nano-Se replicates as compared to normal control. Whereas the challenged replicate detailed hypoproteinemia, hypoglobulinemia and hypoalbuminemia in the control group as compared with the non-challenged normal control. While the Se and nano-Se challenged replicates evidenced hyerproteinemia as compared to control challenged or none.

On looking to some enzymatic assay as prescribed in Table 5, non-challenged groups monitored a significant increase in serum levels of ALT, AST, ALP and LDH in Se replicate while, the nano-Se replicate visualized untouched as compared to the normal control. On the other side the challenged replicates seemed nearly normal in nano-Se group when compared with normal non-challenged replicates while the Se and control group revealed major upsurge in those enzymes as

Table 3
Changes in growth performance of Nile tilapia, *O. niloticus* (means ± SE) fed varied forms of dietary Se for 9 weeks.

Parameters	Groups			
	CT	Se	Nano-Se	P- value
Initial BW (g)	33.29 ± 0.54	33.53 ± 0.64	33.12 ± 0.42	0.962
Final BW (g)	53.17^{b} ± 0.69	52.72^{b} ± 0.73	61.04^{a} ± 1.09	0.013
BW gain (g)	19.88^{b} ± 0.27	19.89^{b} ± 0.31	27.92^{a} ± 0.25	0.015
Specific growth rate	31.55^{b} ± 0.89	30.46^{b} ± 0.65	44.31^{a} ± 1.02	0.012
Feed consumption (g)	60.32 ± 0.19	60.54 ± 0.19	61.09 ± 0.19	0.783
Feed conversion ratio	3.03^{a} ± 0.09	3.04^{a} ± 0.08	2.18^{b} ± 0.04	0.009

Evaluates with dissimilar superscript capital differ significantly at $p < 0.05$, Se = sodium selenite, nano-Se = nano-selenium.

Table 4
Changes in hematological parameters of Nile tilapia, *O. niloticus* (means ± SE) fed varied forms of dietary Se for 9 weeks and post-challenge with *S. iniae.*

Parameters	Groups						P- value
	Non-challenged replicates			Challenged replicates			
	CT	Se	Nano-Se	CT	Se	Nano-Se	
RBCs (10^6/ml)	1.39^{a} ± 0.11	1.31^{a} ± 0.13	1.42^{a} ± 0.09	0.91^{c} ± 0.08	1.19^{b} ± 0.08	1.29^{a} ± 0.08	0.000
Hb (g/dl)	8.00^{a} ± 0.15	8.02^{a} ± 0.23	8.04^{a} ± 0.19	6.01^{c} ± 0.07	7.42^{b} ± 0.09	7.96^{a} ± 0.14	0.000
Ht (%)	27.56^{a} ± 0.91	27.89^{a} ± 0.70	28.05^{a} ± 0.82	24.32^{d} ± 0.54	25.95^{c} ± 0.57	26.99^{c} ± 0.65	0.000
MCV (fl)	198.27^{b} ± 12.32	212.90^{b} ± 15.09	197.54^{b} ± 13.12	267.25^{a} ± 12.98	218.06^{b} ± 17.01	209.22^{b} ± 14.19	0.03
MCH (pg)	57.55^{a} ± 2.61	61.22^{a} ± 3.25	56.62^{a} ± 2.04	66.04^{a} ± 2.98	61.32^{a} ± 3.10	61.71^{a} ± 2.11	0.201
MCHC (%)	29.03^{a} ± 0.86	28.76^{a} ± 0.93	28.66^{a} ± 0.74	24.71^{b} ± 0.79	28.59^{a} ± 0.46	29.49^{a} ± 0.62	0.013
TLC (10^3/µl)	29.11^{c} ± 0.49	30.36^{c} ± 1.32	33.98 ^{b} ± 0.96	42.15^{a} ± 2.18	39.26^{a} ± 0.87	33.83^{b} ± 1.11	0.000
LYM (10^3/µl)	17.51^{b} ± 1.38	17.83^{b} ± 1.66	21.07^{a} ± 0.42	21.65^{a} ± 2.05	21.19^{a} ± 1.43	18.12^{b} ± 0.87	0.000
HET (10^3/µl)	8.89^{c} ± 1.07	9.38^{c} ± 0.76	9.47^{c} ± 0.61	17.61^{a} ± 1.98	14.16^{a} ± 1.06	11.07^{b} ± 0.68	0.000
EOS (10^3/µl)	0.33^{a} ± 0.09	0.31^{a} ± 0.03	0.34^{a} ± 0.05	0.34^{a} ± 0.10	0.33^{a} ± 0.04	0.33^{a} ± 0.02	0.316
MON (10^3/µl)	2.04^{c} ± 0.25	2.51^{b} ± 0.11	2.81^{b} ± 0.02	1.87^{c} ± 0.28	3.29^{a} ± 0.35	4.01^{a} ± 0.42	0.000
BAS (10^3/µl)	0.34^{b} ± 0.01	0.33^{b} ± 0.02	0.29^{b} ± 0.01	0.68^{a} ± 0.04	0.29^{b} ± 0.02	0.30^{b} ± 0.01	0.005

Evaluates with dissimilar superscript capital differ significantly at $p < 0.05$, Se = sodium selenite, nano-Se = nano-selenium, RBCs = erythrocytes, Hb = hemoglobin, Ht = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, TLC = total leukocyte count, LYM = lymphocytes, HET = heterophils, EOS = eosinophils, MON = monocytes,BAS = basophils.

compared to normal unchallenged group.

On inspecting creatinine as one from the most common important renal functions tests (Table 5), the non-challenged replicates publicized significant increase of serum creatinine in Se group without any change in the nano-Se group when matched with the normal control. The challenged groups displayed enormous escalation in the control and Se groups as compared to the normal non-challenged group. The challenged nano-Se group emphasized nearly normal creatinine level as the normal non-challenged control group.

3.4. Effect of varied form of Se on antioxidant status of Nile tilapia pre- and post-challenge with S. iniae infection

Examining of antioxidants as tabulated in Table 6 prescribed significant lessening in the activities of GPx, SOD and CAT with upturn in MDA in Se groups either challenged or none with enormous effect in the challenged replicates as matched to normal control. On the contrary, the nano-Se group in challenged and non-challenged detailed upturn in the activities of GPx, SOD and CAT with lessening in MDA with a potent effect in non-challenged replicates when matched with normal non-

Table 5
Changes in biochemical parameters of Nile tilapia, *O. niloticus* (means ± SE) fed varied forms of dietary Se for 9 weeks and post-challenge with *S. iniae*.

Parameters	Groups						P- value
	Non-challenged replicates			Challenged replicates			
	CT	Se	Nano-Se	CT	Se	Nano-Se	
ALT (U/l)	21.64^{c} ± 0.27	29.43^{b} ± 0.41	12.78^{c} ± 0.39	43.76^{a} ± 0.57	43.98^{a} ± 0.92	22.32^{c} ± 0.35	0.000
AST (U/l)	57.11^{c} ± 1.31	68.88^{b} ± 1.27	46.63^{c} ± 0.87	84.00^{a} ± 0.98	84.24^{a} ± 1.02	58.39^{c} ± 0.64	0.000
ALP (U/l)	26.65^{c} ± 0.60	31.76^{b} ± 0.76	15.96^{c} ± 0.41	67.13^{a} ± 2.23	69.00^{a} ± 2.09	25.81^{c} ± 0.29	0.000
LDH (U/l)	143.00^{c} ± 3.11	176.54^{b} ± 3.75	137.45^{c} ± 2.58	197.37^{a} ± 4.25	201.12^{a} ± 4.08	124.23^{c} ± 2.63	0.000
Creatinine (mg/dl)	0.43^{c} ± 0.014	0.59^{b} ± 0.021	0.45^{c} ± 0.017	1.03^{a} ± 0.11	1.04^{a} ± 0.10	0.47^{c} ± 0.018	0.000
Total proteins (g/dl)	2.98^{c} ± 0.29	3.72 ^{ab} ± 0.22	4.36^{a} ± 0.31	2.01^{d} ± 0.11	3.13^{b} ± 0.33	4.22^{a} ± 0.48	0.000
Albumin (g/dl)	1.29^{a} ± 0.11	1.32^{a} ± 0.03	1.32^{a} ± 0.06	0.91^{b} ± 0.07	1.02 ^{ab} ± 0.07	1.25^{a} ± 0.08	0.427
Globulins (g/dl)	1.69^{c} ± 0.06	2.40^{b} ± 0.09	3.04^{a} ± 012	1.10^{d} ± 0.10	2.11^{c} ± 0.14	2.97^{a} ± 0.23	0.000

Evaluates with dissimilar superscript capital differ significantly at $p < 0.05$, Se = sodium selenite, nano-Se = nano-selenium, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, LDH = lactate dehydrogenase.

Table 6
Changes in antioxidants biomarkers of Nile tilapia, *O. niloticus* (means ± SE) fed varied forms of dietary Se for 9 weeks and post-challenge with *S. iniae.*

Parameters	Groups						P- value
	Non-challenged replicates			Challenged replicates			
	CT	Se	Nano-Se	CT	Se	Nano-Se	
GPx (μmol/mg)	16.28^{b} ± 0.48	13.65^{c} ± 0.53	23.76^{a} ± 0.82	10.12^{d} ± 0.32	10.65^{d} ± 0.41	21.23^{a} ± 0.54	0.000
SOD (U/l)	0.055^{b} ± 0.002	0.031^{c} ± 0.002	0.186^{a} ± 0.005	0.020^{d} ± 0.001	0.019^{d} ± 0.001	0.165^{a} ± 0.004	0.000
CAT (U/l)	16.52^{b} ± 0.48	10.23^{c} ± 0.36	38.21^{a} ± 1.00	6.06^{d} ± 0.23	6.01^{d} ± 1.00	36.18^{a} ± 1.52	0.000
MDA (nmol/ml)	193.66^{b} ± 2.25	221.54^{b} ± 3.14	100.03^{c} ± 2.11	265.96^{a} ± 4.46	267.08^{a} ± 4.61	103.17^{c} ± 2.06	0.000

Evaluates with dissimilar superscript capital differ significantly at $p < 0.05$, Se = sodium selenite, nano-Se = nano-selenium, GPx = Glutathione peroxidase, SOD = superoxide dismutase, CAT = catalase, MDA = malondialdehyde.

challenged replicate. The control and Se challenged replicate exposed the same changes.

3.5. Effect of varied forms of Se on immune parameters of Nile tilapia pre- and post-challenge with S. iniae infection

Regarding to the immune status through determination of immunoglobulin type M, serum lysozyme and nitric oxide as visualized in Table 7, reported significant decrease of them in Se groups either challenged or none as compared with control group. Nano-Se groups documented significant increase in either challenged or non-challenged group when matched with the normal non-challenged control group. The control and Se challenged replicate exposed the same changes.

3.6. Effect of varied form of Se on relative expression of immune and antioxidant related genes of Nile tilapia pre- and post-challenge with S. iniae infection

Analysis of the immune and antioxidant genes expression by RT-PCR. The mean mRNA expression levels of spleen IgM and LYZ or liver SOD and GPx were lessening in Se replicates either challenged or none also, in CT challenged replicate. Where, in the nano-Se replicates, the genes expression rate were pointedly better when judged with the control non-challenged replicate (Figs. 1–4).

3.7. Effect of varied form of Se on cumulative mortality of Nile tilapia after challenge with S. iniae infection

Nile tilapias challenged by *S. iniae* disclosed substantial expansion in the percentage of mortality in CT challenged fish (93.33%), followed by tilapias supplemented with Se (73.33%), whereas the lowermost one was observed at fish supplemented by nano-Se (26.66%) (Fig. 5). The mortalities have been stopped from the 5th, 12th and 14th days in nano-Se, Se and CT respectively.

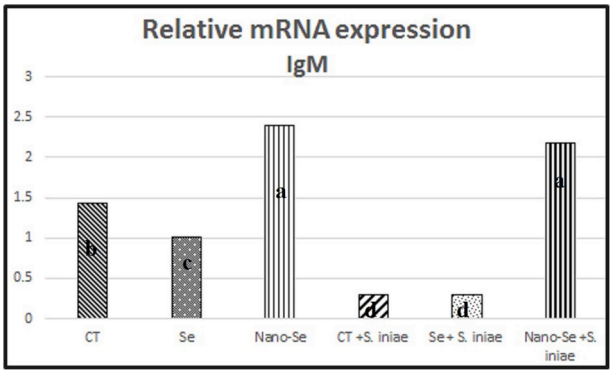

Fig. 1. Influence of varied form of dietary Se supplemented to Nile tilapia, *O. niloticus* for 9 weeks on the relative expression of spleen immune-related gene (IgM) pre- and post-challenge with *S. iniae*.

4. Discussion

Selenium is a crucial trace element for regular physiological run of growing fish. Likewise, its own a forceful antioxidant, immunomodulation and antimicrobial efficiency [43]. Tilapias supplemented with nano-Se diet showed the highest FBW, BW gain and SGR with lowest FCR compared to Se and a basal diets. This finding shows that nano-Se are the most bioavailable sources of selenium for tilapia's growth. This enhancement of the growth measurements conformed to nano-Se raised the intestinal protein contents in epithelial cells instigate well metabolism of feed constituents subsequent in higher growth [44].

Serum enzymes activities supply critical report to relief in fish health valuation [45]. Modifications in blood biochemical levels are

Table 7
Changes in innate immunity biomarkers of Nile tilapia, *O. niloticus* (means ± SE) fed varied forms of dietary Se for 9 weeks and post-challenge with *S. iniae.*

Parameters	Groups						P- value
	Non-challenged replicates			Challenged replicates			
	CT	Se	Nano-Se	CT	Se	Nano-Se	
Nitric oxide (nmol)	46.63^{b} ± 0.78	34.14^{c} ± 0.41	77.58^{a} ± 2.34	32.09^{c} ± 0.41	33.00^{c} ± 0.41	73.51^{a} ± 2.19	0.000
IgM (μg/ml)	26.32^{b} ± 0.76	15.64^{c} ± 0.51	37.92^{a} ± 1.25	13.56^{c} ± 0.45	14.87^{c} ± 0.49	29.18^{b} ± 0.67	0.000
lysozyme (μg/ml)	22.97^{b} ± 0.79	16.15^{c} ± 0.54	30.13^{a} ± 1.49	15.43^{c} ± 0.74	17.05^{c} ± 0.38	28.47^{a} ± 1.64	0.000

Evaluates with dissimilar superscript capital differ significantly at $p < 0.05$, Se = sodium selenite, nano-Se = nano-selenium, IgM = immunoglobulin M.

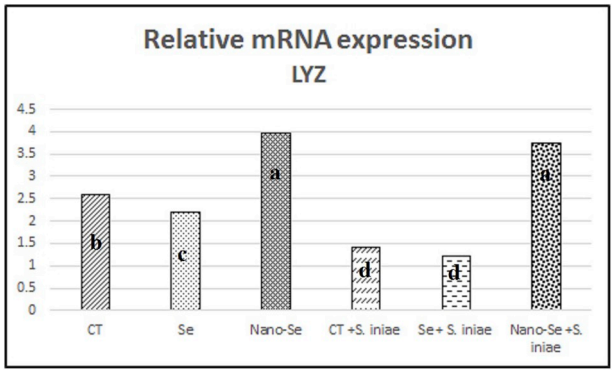

Fig. 2. Influence of varied form of dietary Se supplemented to Nile tilapia, *O. niloticus* for 9 weeks on the relative expression of spleen immune-related gene (LYZ) pre- and post-challenge with *S. iniae*.

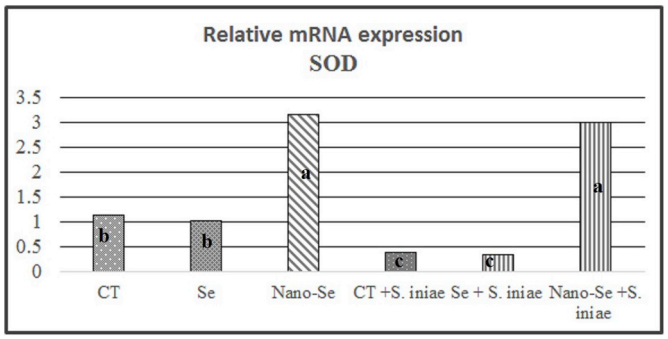

Fig. 3. Influence of varied form of dietary Se supplemented to Nile tilapia, *O. niloticus* for 9 weeks on the relative expression of liver antioxidant-related gene (SOD) pre- and post-challenge with *S. iniae*.

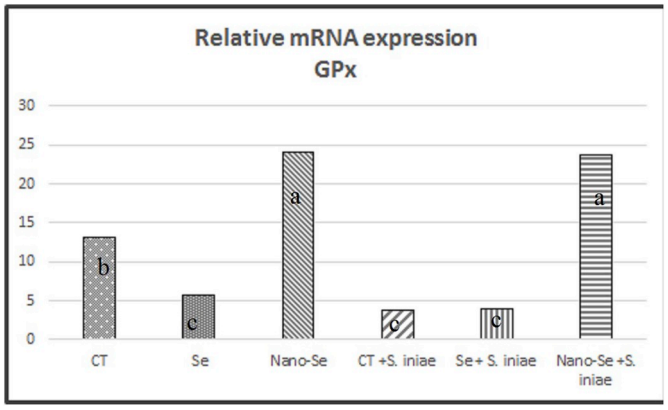

Fig. 4. Influence of varied form of dietary Se supplemented to Nile tilapia, *O. niloticus* for 9 weeks on the relative expression of liver antioxidant-related gene (GPx) pre- and post-challenge with *S. iniae*.

frequently the first measureable for a retorts to ecological variation. It could be suggestive of occurrence of stress aspects such as toxic chemicals, overcrowding and or error in aquaculture processes [46]. The non-challenged replicate monitored a significant increase in serum levels of ALT, AST, ALP, LDH and creatinine in tilapias supplemented with Se. These amendments in the activity of those parameters may be stress prompted and related to tissue mutilation [47], as liver is the core place of Se accumulation in fish [48]. The non-challenged nano-Se replicate visualized untouched of those parameters.

The rise of liver enzymes and creatinine within *S. iniae* challenged control replicates could owed to necrosis and degeneration of its tissues during septicemia [49,50]. Nano- Se challenged replicates emphasized

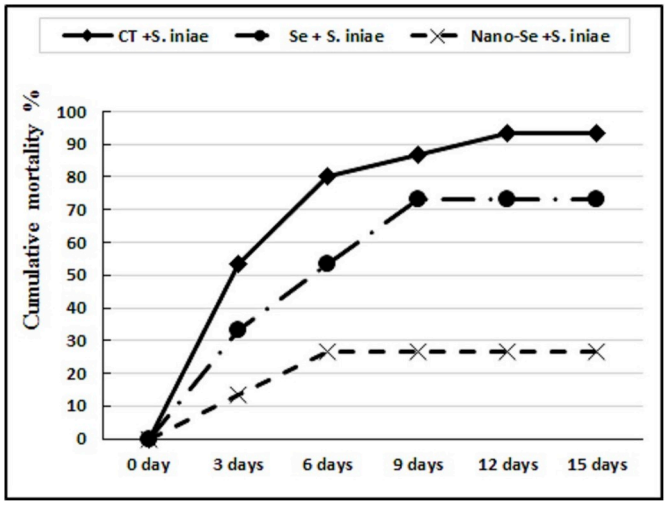

Fig. 5. Cumulative mortality (%) of Nile tilapia, *O. niloticus* fed diets supplemented with varied forms of dietary Se for 10 weeks (post-challenged with *S. iniae* at the start day of 9th week).

nearly normal level due to safety and shield of nano-Se on tilapia and or counteract of infection, similarly in common carp [9]. The influence of Se is subjected to its level and chemical structure. There is an extremely narrow range among essential and the toxic levels of dietary Se in fish [3,51]. The increase in these parameters proposes greater toxicity of Se in an inorganic than nano form. Analogous records were established the toxicity in Common Barbel and African catfish nourished 0.3 and 0.5 mg kg^{-1} Se correspondingly propositioning liver and kidney hazards [47,52].

Se is critical for the polite running of the antioxidant enzymes, which shield against oxidative stress [53]. Maintenance of cellular oxidative encompass on the antioxidant shield system and incorporating enzymes as GPx, CAT and SOD which can be working as indicators of oxidative stress and vital for sifting the reactive oxygen species (ROS) [54,55]. The antioxidant influence of Se is returned to its assimilation in selenocysteine, which is a part of the effective center of the GPx. GPx has a protective role as anti-oxidative radicals in body and augments the body's cell endurance as immune cells anti peroxidative injury [56]. Level of MDA is reflected as an index of lipid peroxidation consequences and the lessen MDA level is correlated to longer shelf life and depend on the antioxidants [57]. Tilapias nourished with 0.7 mg kg^{-1} Se prescribed significant lessening in the activities of GPx, SOD and CAT with upturn in MDA in Se groups either challenged or none with enormous effect in the challenged replicates. The alike approach to its gene expression. NO is a vigorous signaling element involved in the ruling of assorted pathophysiological ways in immunological systems [58], it also implicated in lipid peroxidation mediated injury [59]. Superoxide combine with NO, abetting in the formation of peroxynitrite. Which assisting ROS in destroying challenged pathogen [60].

On the contrary, the nano-Se group in challenged and non-challenged detailed upturn in the activities of GPx, SOD, CAT and NO with lessening in MDA with potent effect in non-challenged replicates when matched with normal non-challenged replicate. Also, these results are subsequent with the increased regulation of CAT and GPx gene expression in nano-Se tilapias. Finally, these results signifying that nano-Se form is more potent than Se in establishment the antioxidant defense ability in tilapias against oxidative stress as infection [61,62]. In harmony, other studies exhibited that nano-Se could augment activity of GPx, antioxidant ability, and oxidative stress endurance in varied fish species; yellowtail kingfish [63] common carp [10,64,65].

In vertebrates involving fish, the blood is the most recurrently studied tissue in attempts to ascertain their health or physiological status.

Hence, health status as oxygen delivery aptitude has been straight concluded by situation of hematological indicators [66]. The previous data established potent antioxidant capacity of the nano-Se which assumed their potency to increase the constancy of erythrocytes membranes and their endurability by guarding them against free radicals, avoiding membrane damage, hemolysis and anemia [10]. Existence of macrocytic hypochromic anemia in CT *S. iniae* challenged replicates represented by decline in RBCs number, Hb, Ht and MCHC with a raise of MCV could attained to subsequent hemorrhage located in septicemic streptococcosis [67,68]. Anemia in Se challenged replicates in a lesser degree while, nano-Se remain unchanged. This may explain the task of nano- Se in protecting erythrocyte from hemolysis either by influential antioxidant impact [61,62] or antimicrobial efficiency counteract infection [43].

Selenoproteins complex, specifically GPx, may guard heterophils and macrophages from superoxide radicals [69]. Our analysis, using of nano-Se denotes raise in numbers of leukocyte, lymphocytes and monocytes in non-challenged replicates with monocytosis in challenged one compared to the normal control that could augment of fish vigor condition. In fish, it has been testified that nano-Se is more quickly absorbed, and more potent than sodium selenite in designates of bioavailability and achieves on health [63]. Tilapias challenged by *S. iniae* represented lymphocytic and heterophilic leukocytosis, that aid in phagocytosis of the challenged pathogen [70]. This somewhat harmonizes with the results obtained by (Badr et al., 2012) [50] who revealed heterophilic leukocytosis in tilapias challenged by *S. iniae*. Using of nano-Se more compelling than Se in relieving this near the normal limits [10].

Enhancement of innate immune biochemical factors as total proteins, LYZ defy against S. iniae bacterial pathogen in tilapias [71]. Immunomodulating agent's expansion serum LYZ by rising the number of phagocytes producing LYZ and or its amount synthesized benefit in pathogen invaders elimination [72]. Augmented LYZ and its gene expression in nano-Se could be accompanying with enlarged leukocytic counts, principally heterophils the foremost LYZ producer [10]. IgM can induce an effectual specific humoral reply from B-lymphocytes against varied pathogen [28,73]. Decrease of them in Se replicates either challenged or none with arise in nano-Se replicates verified immunomodulating effect of the later [10]. The alike approach was in IgM gene expression. The serum total proteins are primarily manufactured by liver cells, exploited as a wide gauge of health, immune capability and stress in fish. Also absorbed Se is compelled to albumin aiding selenoprotein manufacture [74]. The decline of proteins and globulins in *S. iniae* replicate could due to its immunosuppression or kidney and liver dysfunction impact [50].

The antimicrobial guard of fish hang on the immune status reaction to these exposed to infections. Therefore, the ultimate test of immune system job with the extreme resistance to the challenge pilot [75]. The testified in this analysis, upon addition of nano-Se generate more antioxidant defense, as well as innate immune modules than Se [10,43].

5. Conclusions

From the previous results, we can conclude that using of Se 0.7mg/kg^{-1}induce immunosuppressive, antioxidant, liver and kidneys negative impacts on tilapias where the same dose of nano-Se produce a potent immunomodulating and antioxidant effects. Also was attending in counteract the serious impacts induced by *S. iniae* challenge.

Conflicts of interest

None.

Acknowledgment

The authors thank Dr. Abdou A A, Department of Pharmaceutics, Faculty of pharmacy, Al-Azhar University for his help in manufacture of nano-Se.

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