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Miswak (*Salvadora persica*) dietary supplementation improves antioxidant status and nonspecific immunity in Nile tilapia (*Oreochromis niloticus*)

Mohamed A. Lebda[a], Waleed N. El-Hawarry[b], Ramy M. Shourbela[b], Ali H. El-Far[c,*], Ramadan S. Shewita[d], Shaker A. Mousa[e]

[a] *Biochemistry Department, Faculty of Veterinary Medicine, Alexandria University, Egypt*
[b] *Animal Husbandry and Animal Wealth Department, Faculty of Veterinary Medicine, Alexandria University, Egypt*
[c] *Biochemistry Department, Faculty of Veterinary Medicine, Damanhour University, Egypt*
[d] *Nutrition and Veterinary Clinical Nutrition Department, Faculty of Veterinary Medicine, Alexandria University, Egypt*
[e] *Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, 12144, USA*

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) is the most common aquaculture fish, but is exposed to various pollutants and may be susceptible to infectious diseases due to reduction in their antioxidant status and immune defense. Therefore, researchers have tried to find feed supplements of natural origin to increase the health status of fish and decrease the incidence of drug resistance. The current study was conducted to investigate the effect of dietary supplementation of Miswak (*Salvadora persica,* SP) on Nile tilapia. Fish were randomly allocated into four experimental groups (30 fish each); the control fish were fed on the basal commercial diet and the SP-treated groups were fed basal diet supplemented with different concentrations 0.5, 1, and 2% for 30 days. The SP supplementations had no significant effects of SP on fish growth performance traits and lipid profiles but augmented the serum protein and globulin levels. The SP significantly improved the hepatic antioxidant status through the significant decrease of malondialdehyde (MDA) and the increases of reduced glutathione (GSH) levels and the activities of total superoxide dismutase (T.SOD), catalase (CAT) and glutathione peroxidase (GPx), especially in the SP 1% group, while glutathione S-transferase (GST) activities were significantly increased due to SP in a dose-dependent manner. The same results were obtained for the mRNA expression of *CAT* and *GPx*. Regarding the nonspecific immune status of the fish kidneys, SP, especially SP1, significantly increased interleukin-1 beta (*IL-1β*) and interferon-gamma (*INF-γ*). The data of the present study revealed the protective effect of SP on Nile tilapia health status. Therefore, SP can be considered as a promising feed additive for Nile tilapia.

1. Introduction

Aquaculture is one of the upcoming agriculture industries. Nile tilapia (*Oreochromis niloticus*) is the most commercially used aquaculture fish [1]. However, aquaculture production has been threatened by various industrial developments that cause pollution of the aquatic water and different aquatic diseases [2]. Also, the intensified culture systems were considered as an obstacle of sustainable aquaculture development because the risk of infectious disease occurrence is high [3]. In addition, the intensive culture system of fish production encourages the risk of infectious diseases, major stock mortality, and economic losses [4].

The use of chemical drugs is one of the most applied methods to counteract the risk of infectious diseases in fish farms, however, their usage is limited because drug residues accumulate in aquatic products, drug resistance develops, and there are undesirable environmental effects [5]. Many traditional approaches have been developed to combat infectious diseases and enhance fish growth performance in aquaculture. Natural products are of great interest in aquaculture applications to enhance the immune systems of fish and thereby increase their capacity for disease resistance and promote growth performance [6]. The application of medicinal plants in fish farms has focused on their feeding attraction, growth promotion, and immune system activation [7]. Furthermore, medicinal plants are of great interest as potential therapeutics for modification of immune response and control of fish diseases [8]. Different herbs have been used in aquaculture as an effective antimicrobial, improved nourishment substances, and growth enhancers [9].

* Corresponding author.
E-mail address: ali.elfar@damanhour.edu.eg (A.H. El-Far).

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Table 1
Feed ingredients and nutrient composition of the basal diet (g/kg).

Feed ingredients	g/kg diet
Soybean oil meal (CP 47%)	276
Ground yellow corn	274
Fish meal (CP 60%)	180
Wheat bran	130
Rice bran (with germ)	125
Di-calcium phosphate	10
DL-Methionine	0.5
Choline chloride	0.5
Vitamin mix[a]	1
Mineral mix[b]	1
Binders[c]	2
Nutrient composition	**(g/kg)**
Dry matter (DM)	886
Metabolizable energy[d] (kcal/kg diet)	2790.8
Ether extract	32
Crude protein (CP)	300
Crude Fiber (CF)	46
Ash	80

[a] Each 1 kg contains (mg): vitamin B_1: 1000, vitamin B_2: 5000, vitamin B_6: 15000, vitamin B_{12}:1.0, nicotinic acid: 30000, biotin: 50, folic acid: 1000, pantothenate: 10000, vitamin E: 10000, vitamin K3: 2000 and contains (IU): vitamin A: 12000000, vitamin D3: 2000000 IU, and choline: 125000.
[b] Each 1 kg contains (mg): $FeSO_4$ (15000), $ZnSO_4$ (25.000), $MnSO_4$ (30000), $CuSO_4$ (5000), CaI_2 (500), and cobalt (50).
[c] Binder: Sodium carboxymethylcellulose (high viscosity).
[d] Calculated per National Research Council (NRC) [54].

The antioxidant status of fish has been improved by dietary supplementation with numerous natural products. Sayed et al. [10] stated the hepatoprotective effect of *Spirulina platensis* against lead-induced oxidative stress and genotoxicity in catfish; *Clarias gariepinus*. Gobi et al. [11] stated that selenium significantly increased the superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPx, EC 1.11.1.9), and glutathione S-transferase (GST, EC 2.5.1.18) antioxidant enzymes in the gill and liver tissues of *O. mossambicus*. Also, β-1, 3 glucan binding protein based selenium nanowire improved the immune status of *Cyprinus carpio* [12]. In the same manner, *Bacillus licheniformis* Dahb1 as a probiotic improved the growth performance, mucus, and serum immune parameters, and the antioxidant status in *O. mossambicus* [13].

Miswak (*Salvadora persica* L., SP) tree is found in many Asian countries. Different parts of this tree were introduced in folk medicine mainly for oral hygiene [14]. SP has numerous pharmacological activities including antioxidant [15], anti-inflammatory and anti-ulcerogenic [16], antibacterial [17], and antifungal [18]. Also, feeding SP to the common carp improved the survival rate and immune functions [19]. However, there is little literature about the effects of SP on Nile tilapia. Therefore, this study was conducted to evaluate the efficacy of SP on growth performance, antioxidant status, and nonspecific immune response in tilapia.

2. Material and methods

2.1. Ethics statement

The experiment was carried out with reference to the guidelines and recommendations of EU Directive 2010/63/EU for animal experiments and the Canadian Council on Animal Care and approved by the Animal Care and Welfare Committee of Alexandria University, Egypt.

Table 2
Primer sequences and cycling conditions.

Target genes	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
				Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
Elongation factor 1-α (*EF-1α*)	F: CCTTCAACGCTCAGGTCATC R: ATGGGAAGTCTTCCACTGTC	50 °C 30 min	94 °C 5 min	94 °C 15 s	62 °C 30 s	72 °C 30 s	94 °C 1 min	62 °C 1 min	94 °C 1 min
Hepatic catalase (*CAT*)	F: TCCTGAATGAGGAGGAGCGA R: ATCTTAGATGAGGCGGTGATG				60 °C 30 s			60 °C 1 min	
Hepatic glutathione peroxidase (*GPx*)	F: CCAAGAGAACTGCAAGAACGA R: CAGGACACGTCATTCCTACAC				60 °C 30 s			60 °C 1 min	
Renal interleukin-1 beta (*IL-1β*)	F: GCTGGAGAGTGCTGTGGAAGAACATATAG R: CCTGGAGCATCATGGCGTG				62 °C 30 s			62 °C 1 min	
Renal interferon-gamma (*INF-γ*)	F: AAGAATCGCAGCTCTGCACCAT R: GTGTCGTATTGCTGTGGCTTCC				60 °C 30 s			60 °C 1 min	

Table 3
Effect of dietary supplementation of Miswak (*Salvadora persica*) on growth performance parameters in Nile tilapia.

Parameters	Groups			
	Control	SP0.5	SP1	SP2
Initial BW (g)	15.58 ± 0.57	15.33 ± 0.08	15.52 ± 0.29	15.38 ± 0.23
Final BW (g)	38.17 ± 3.22	37.75 ± 4.02	37.52 ± 3.90	36.33 ± 4.38
TBG (g)	22.58 ± 3.43	22.42 ± 4.01	22.00 ± 4.06	20.95 ± 4.30
TBG (RTC)	100	99.29	97.43	92.78
SGR	1.49 ± 0.08	1.49 ± 0.08	1.46 ± 0.09	1.42 ± 0.08
TFI (g) per fish	34.88 ± 2.79	34.28 ± 3.64	34.23 ± 3.39	33.05 ± 4.01
FCR	1.56 ± 0.14	1.55 ± 0.12	1.58 ± 0.18	1.60 ± 0.15
PER	2.15 ± 0.18	2.16 ± 0.16	2.13 ± 0.21	2.10 ± 0.19
Initial body length (cm)	9.97 ± 0.23	9.92 ± 0.37	9.93 ± 0.29	10.15 ± 0.34
Final body length (cm)	13.03 ± 0.66	12.92 ± 0.72	12.88 ± 0.68	12.40 ± 1.16
Initial CF	1.58 ± 0.12	1.59 ± 0.18	1.59 ± 0.12	1.48 ± 0.17
Final CF	1.74 ± 0.27	1.77 ± 0.28	1.78 ± 0.32	1.98 ± 0.48

Mean values with different letters in the same row differ significantly at $P < 0.05$.
Values are expressed as means ± standard error.
Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test for multiple comparisons.
SP0.5, fish group received basal diet containing 0.5% of SP; SP1, fish group received basal diet containing 1% of SP; SP2, fish group received basal diet containing 2% of SP.
CF, condition factor; FCR, feed conversion ratio; PER, protein efficiency ratio; SGR, Specific growth rate; TBG, Total body weight gain of fish; TFI, total feed intake.

2.2. Fish, rearing, and feeding

Nile tilapia with an average body weight of 15.4 ± 0.12 g/fish were obtained from private aquaculture at Kafr Elsheikh governorate, Egypt. The fish were transported alive to the Animal Husbandry Department, Faculty of Veterinary Medicine, Alexandria University, Egypt, where they were maintained in tanks provided with chlorine-free tap water with continuous aeration, temperature 28 ± 2 °C, pH 7.5, dissolved oxygen 6.5 mg/L, ammonia 0.01–0.05 mg/L, and 12:12 h light/dark cycle.

The fish were fed on the basal commercial diet composed of ingredients and nutrients as listed in Table 1, at the rate of 2% of body weight for an acclimation period of 2 weeks. Fish were then allotted into three experimental groups in addition to a control group (30 fish each). Control fish were fed on the basal commercial diet, while the treated groups were fed basal diet supplemented with 0.5, 1, and 2% SP for 30 days. Fish were kept under the standard conditions mentioned above.

2.3. Experimental diets and supplement

SP roots were obtained from the local market at Alexandria governorate, Egypt. The roots were air dried and ground to make a fine powder. Three different experimental diets were prepared by mixing 0.5, 1, and 2 g SP powder with 100 g basal diet powder and blended with 30 mL tap water to make a paste. Then pastes were pelleted into fine pellets of 2 mm diameter using a pellet machine. The obtained pellets were air dried at room temperature for 24 h, prepared weekly, and stored at 4 °C for daily use. During the experimental period, a fixed feeding rate of 3% of the fish wet weight per day was supplied [20]. The quantity of feed related to fish weight was adjusted through the bi-weekly weighing of a random sample of 10 fish from each treatment group in the early morning before feeding.

The aqueous extract of SP roots that was used in the current study contained approximately the same antioxidant constituents including lycopene (16.56%), α-linolenic acid (1.77%), oleic acid (1.97%), lycoxanthin (1.61%), and retinoic acid (1.31%) as stated in our previous study of Lebda et al. [16].

2.4. Growth performance

At the start and at the end of the trial, six fish were randomly taken from each group using a scoop net, drained for 30 s, and transferred into a container with enough water to eliminate environmental stress, to evaluate the effect of SP on fish growth performance. The individual body weights (BW) were recorded using a sensitive electronic balance to the nearest 0.1 g, and the whole-body length (cm) of each fish was measured from the anterior of the fish to the end of its tail. Then fish were immediately returned to the original water environment.

The total feed intake (TFI) during the trial was recorded. Total body weight gain of fish (TBG) was calculated as the difference between the initial and final body weights, and the feed conversion ratio (FCR) was calculated according to the method of Degani et al. [21]. Specific growth rate (SGR) and protein efficiency ratio (PER) values were calculated according to the method of Jauncey and Ross [22], while condition factor (CF), which relates fish body length to body weight, was computed according to the method of Higgs et al. [23].

2.5. Sampling

At the end of the trial, five fish were randomly selected from each group and anesthetized using tricaine methanesulfonate by a concentration of 200 mg/L [10], which constitutes 0.02% w/v according to the guidelines for the preparation and use of tricaine methanesulfonate for animal procedures approved by Florida Atlantic University's Institutional Animal Care and Use Committee (IACUC). Blood samples were collected from the caudal vein using a heparinized syringe. Collected blood samples were centrifuged at 3000 × *g* for 10 min at 4 °C to collect clear sera for biochemical analyses.

Liver tissues were removed, washed in chilled saline, frozen quickly in liquid nitrogen, and stored at −80 °C for evaluation of antioxidant status and for RT-PCR analyses. Also, samples from kidney's head were taken for analysis of mRNA expression of interleukin-1 beta (*IL-1β*), and interferon-gamma (*INF-γ*).

2.6. Biochemical analyses

Collected clear sera were separated following blood coagulation and kept at −20 °C until further analyses of total protein, albumin, triacylglycerol (TAG), total cholesterol (T.cholesterol), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), and low density lipoprotein cholesterol (LDL-C) using commercial Biodiagnostic (Giza, Egypt) kits according to the manufacturer's instructions. Serum globulin levels were calculated by subtracting the albumin value from the total protein value of the same sample [24].

2.7. Antioxidant status assessment

Oxidative stress and antioxidant biomarkers were analyzed in liver homogenate 20% (w/v) using cooled 0.1 M phosphate buffer saline; malondialdehyde (MDA) was expressed as nmol/mg protein [25], reduced glutathione (GSH) represented as mmol/mg protein [26], T.SOD activity as U/mg protein [27], CAT as U/mg protein [28], GPx activity as U/mg protein [29], and GST activity as U/mg protein [30]. Protein concentrations of liver homogenates were assessed with the Bradford assay (5000002, Bio-Rad Laboratories, Watford, UK) for standardization of biochemical parameters [31].

2.8. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis

RT-PCR was done to assess the mRNA levels of *CAT*, *GPx*, *IL-1β*, and *INF-γ*. Briefly, total RNA was extracted from liver and kidney's head tissue samples and the quality of the extracted RNA was confirmed using a NanoDrop spectrophotometer, then total RNA was reverse transcribed to first-strand cDNA according to the manufacturer's instructions. The cDNA was used as the template for RT-PCR. The sequences of the primers used are provided in Table 2. The relative fold changes in gene expression between the control and the three experimental groups were calculated using threshold cycle (CT) values against those of the housekeeping (*β-actin*) gene in the same sample using the $2^{-\Delta\Delta CT}$ method [32].

2.9. Statistical analysis

The data were statistically analyzed with GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) using one-way ANOVA with Tukey's Multiple Comparison Test. $P < 0.05$ was considered statistically significant. Results are shown as means ± standard error.

3. Results

3.1. Growth performance

The growth performance parameters of Nile tilapia fed diets with different levels of SP are shown in Table 3. The data showed non-significant differences in the growth performance parameters including final body weight, TBG, total feed intake, FCR, SGR, PER, and CF between all treated groups fed a diet with different levels of SP at 0.5, 1, or 2% and the control group.

3.2. Serum biochemical assessment

The data in Fig. 1 revealed that the serum levels of total protein, albumin, and globulins were statistically increased in SP1 group ($P < 0.01$) when compared with the control. Also, fish in SP1 had improved levels of total protein, albumin, and globulins that were higher than levels for the SP0.5 and SP2 groups.

Supplementation with SP caused no significant changes on the serum lipid fractions in fish as shown in Fig. 2 except on HDL-C, which significantly increased ($P < 0.05$) in SP2 compared with the control group.

3.3. Hepatic oxidative stress and antioxidant status

The hepatic MDA concentration, a marker of oxidative damage, was significantly decreased in SP0.5 ($P < 0.001$), SP1 ($P < 0.001$), and SP2 ($P < 0.01$) groups relative to the control (Fig. 3A). Furthermore, the hepatic GSH levels showed significant increases in SP1-treated fish ($P < 0.01$) and SP2 ($P < 0.05$) compared with control fish (Fig. 3B). Regarding the enzymatic activities of antioxidant, T.SOD, CAT, and GPx (Fig. 3C–E) were significantly increased ($P < 0.001$) in all SP-treated groups with respect to control. In addition, fish in SP1 had the highest CAT and GPx activities compared with SP0.5 and SP2. GST activities in SP-treated groups were significantly increased in comparison with the control group in a dose-dependent manner (Fig. 3F).

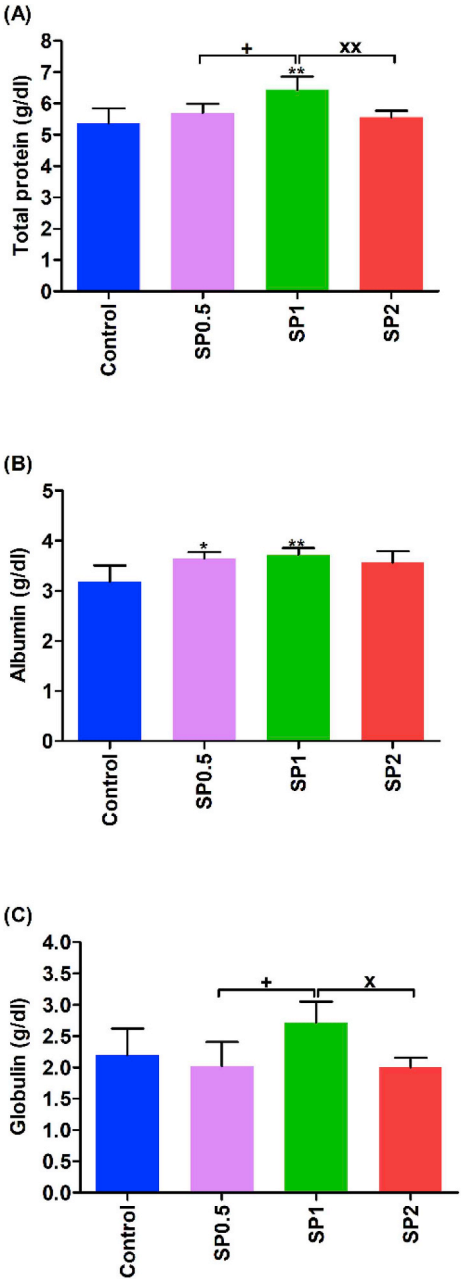

Fig. 1. Serum levels of (A) total protein (g/dl), (B) albumin (g/dl), and (C) globulin (g/dl). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control. $^{+}P < 0.05$ vs. SP0.5. $^{x}P < 0.05$ and $^{xx}P < 0.01$ vs. SP1. Statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test for multiple comparisons. SP0.5, fish group received basal diet containing 0.5% of SP. SP1, fish group received basal diet containing 1% of SP. SP2, fish group received basal diet containing 2% of SP.

3.4. Gene expression

The mRNA expressions of *CAT* and *GPx* are illustrated in Fig. 4. The expression folds of both genes were significantly ($P < 0.001$) increased in all SP-fed groups compared with the control group. Interestingly, fish in the SP1 group showed the greatest expression folds for both genes (~2.5-fold) as compared with SP0.5 and SP2 groups.

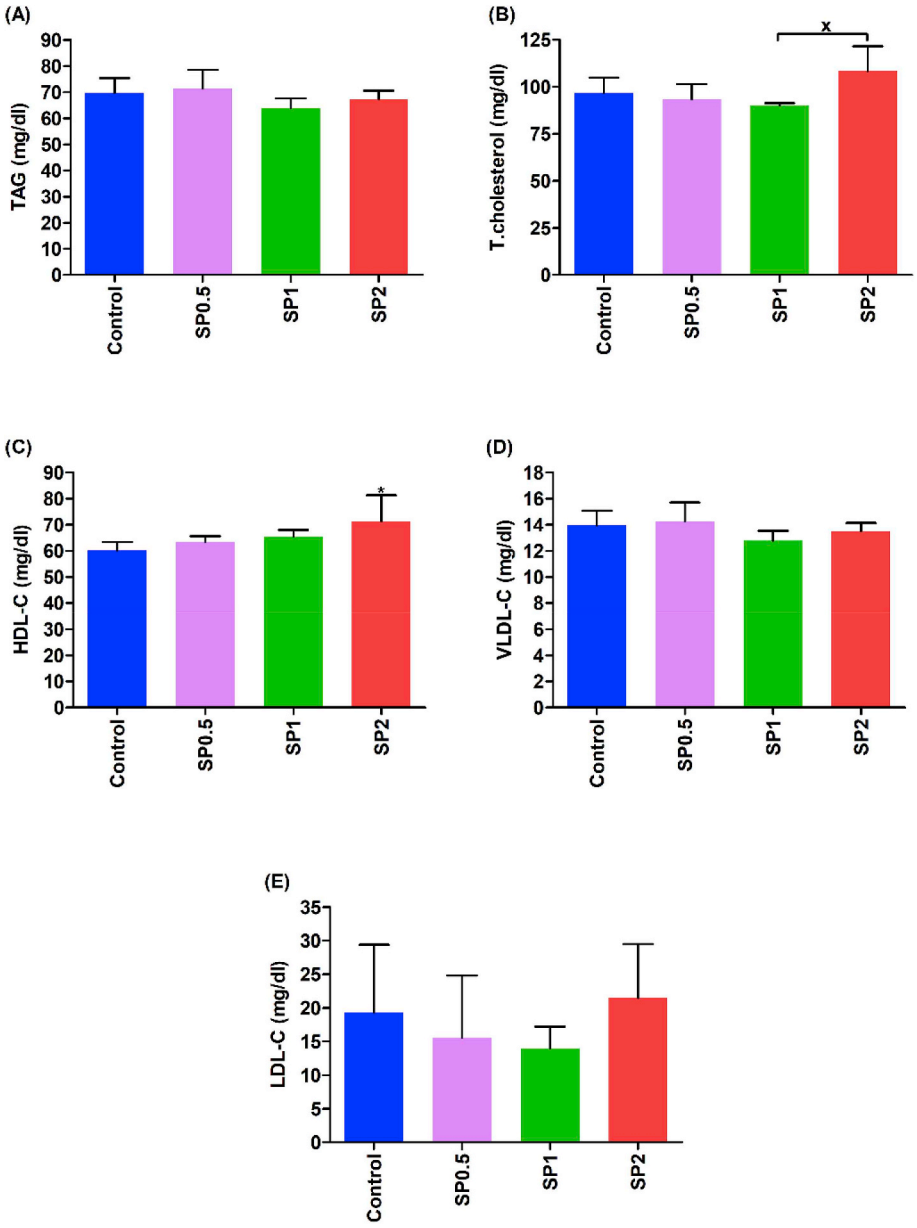

Fig. 2. Serum levels of (A) TAG (mg/dl), (B) T.cholesterol (mg/dl), (C) HDL-C (mg/dl), (D) VLDL-C (mg/dl), and (E) LDL-C (mg/dl). $^{*}P < 0.05$ vs. control. $^{x}P < 0.05$ vs. SP1. Statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test for multiple comparisons. SP0.5, fish group received basal diet containing 0.5% of SP. SP1, fish group received basal diet containing 1% of SP. SP2, fish group received basal diet containing 2% of SP. TAG, triacylglycerol. T.cholesterol, total cholesterol. HDL-C, high density lipoprotein cholesterol. VLDL-C, very low-density lipoprotein cholesterol. LDL-C, low density lipoprotein cholesterol.

The relative expression of immune-related genes is represented in Fig. 5 mRNA transcription of *IL-1β* was significantly decreased in the kidney's head of fish fed with 0.5% and 2% SP-containing diet at $P < 0.001$, while 1% SP-fed fish had a significant increase (~1.6-fold) in the transcription of this gene relative to control. Similarly, the expression of *INF-γ* mRNA transcript was non-significantly changed in the SP0.5 group, but significantly decreased ($P < 0.001$) in the SP2 group compared with control. On the other hand, fish in SP1 showed significant increases ($P < 0.001$) of *INF-γ* mRNA expression with respect to control and other SP-treated groups.

4. Discussion

SP feed supplementations to Nile tilapia fish induced no significant differences in the growth performance traits. This might be attributed to the low levels of amino acids and total nitrogenous compound contents in the SP [33]. However, previous studies confirmed the growth promoter effect of SP in Baladi rabbit [34] and poultry [35]. This variation may contribute to the species difference and needs further studies in fish.

In the current study, the fish in the SP1 group had significant improvements of serum total protein, albumin, and globulins. Similar results were observed by Yilmaz et al. [36] who reported that feeding rainbow trout with carvacrol, one of the major SP constituents, improved the levels of serum total protein, albumin, and globulins. It was noticed that the high serum total protein, albumin, and globulins concentrations suggested the enhancement of innate immune response in fish [37]. Also, our results coincide with those of El Rabey et al. [38] who mentioned that pre-treatment of diabetic rats with SP elevated the serum level of immunoglobulins. Increased immunoglobulins levels were associated with elevated serum globulin fraction and subsequent serum total protein, which is indicative of an enhanced immune system.

SP feed supplementations caused non-significant changes on serum lipid fractions in fish. Further investigations are necessary to evaluate the effect of SP on serum lipid profiles. In the same context, Ramadan and Alshamrani [39] stated that SP restored impaired lipid profiles due to stress to their normal levels in rats. Also, SP of Arabic and Indian origins showed significant hypoglycemic and hypolipidemic activities in diabetic rats [40].

The antioxidant status of the liver improved as indicated by a

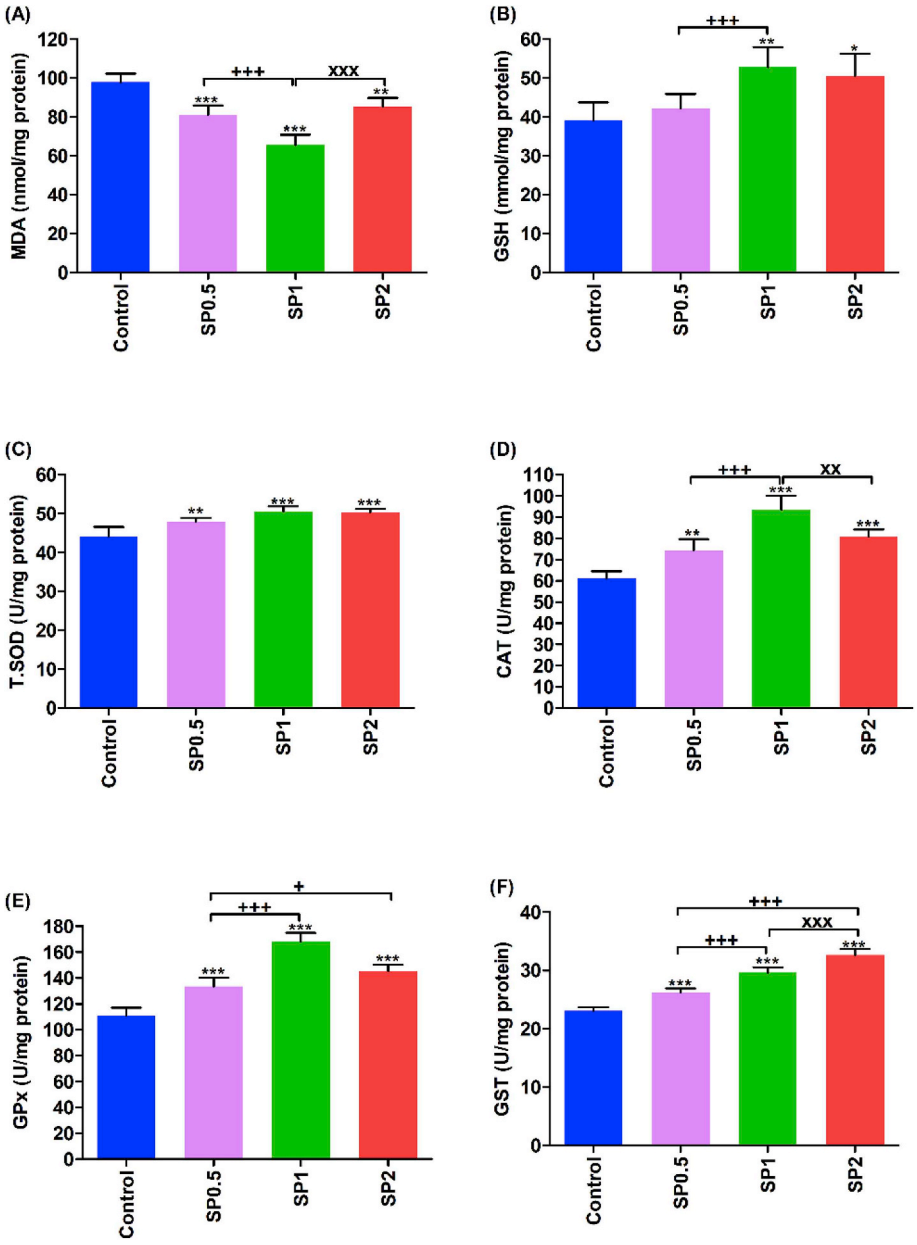

Fig. 3. Hepatic (A) MDA (nmol/mg protein), (B) GSH (mmol/mg protein), (C) T.SOD (U/mg protein), (D) CAT (U/mg protein), (E) GPx (U/mg protein), and (F) GST (U/mg protein). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control. $^{+}P < 0.05$ and $^{+++}P < 0.05$ vs. SP0.5. $^{xx}P < 0.01$ and $^{xxx}P < 0.001$ vs. SP1. Statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test for multiple comparisons. SP0.5, fish group received basal diet containing 0.5% of SP. SP1, fish group received basal diet containing 1% of SP. SP2, fish group received basal diet containing 2% of SP. MDA, malondialdehyde. GSH, reduced glutathione. T.SOD, total superoxide dismutase. CAT, catalase. GPx, glutathione peroxidase. GST, glutathione S-transferase.

significant reduction in MDA and increases in GSH levels and T.SOD, CAT, GPx, and GST activities in the SP-treated group, especially the SP1 group. Also, the same results were obtained for the mRNA expression of *CAT* and *GPx* genes due to SP supplementations, which indicates the powerful antioxidant effect of SP on fish livers.

The antioxidant activity of SP was recorded by Kumari et al. [15] who attributed this effect to the natural antioxidant constituents of SP. The main active principles of SP stem were benzyl isothiocyanate, aniline, carvacrol, benzyl nitrile, naphthalene, and benzaldehyde [41]. Furthermore, Al-Dabbagh et al. [42] suggested that SP extract was rich in total polyphenolic and flavonoid compounds that exhibit potent antioxidant activity. Ibrahim et al. [43] reported the antioxidant activity of SP via hydrogen peroxide scavenging ability and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods and explained this activity was related to the high content of total phenolic and flavonoid compounds.

The increased hepatic CAT expression and activity in SP-treated fish detoxifies H_2O_2 into H_2O and O_2, which protects the liver against oxidative injuries. This is the main role of CAT as a vital member of the enzymatic antioxidant system [44]. Therefore, CAT is considered a potential biomarker for different herbicides intoxication in fish [45]. After SOD enzyme converts the superoxide anion radical to H_2O and H_2O_2, both CAT and GPx catalyze detoxification of H_2O_2 [46]. GPX prevents many molecules from being damaged by the oxidative effect of increasing cellular H_2O_2through conjugation with two GSH molecules, converting them to oxidized glutathione (GSSG) [47].

The renal expression of *IL-1β* and *INF-γ* genes was significantly increased in SP1. IL-1β at a limited level had a critical role in combating infections via activation of innate and adaptive response, however, exaggerated activation of IL-1β was incriminated in the pathogenesis of inflammatory conditions [48]. Inflammation is a part of innate immunity and has a protective role against infection where IL-1β initiates an immune response through inhibition of micro-organisms' colonization, replication, and invasion [49]. In this study, SP upregulated the mRNA level of IL-1β, suggesting its immune-stimulant rather than inflammatory effect. Also, Hong and Secombes [49] revealed that recombinant trout IL-1β therapy induced the mRNA transcription of IL-1β and IL-8, suggesting the immunological response. It was established that SP is rich in carotenoids such as lycopene, retinoic acid, and lycoxanthin [16]. In the same context, lycopene promotes the modulation of the immune response via increasing the production of *IL-1β* [50].

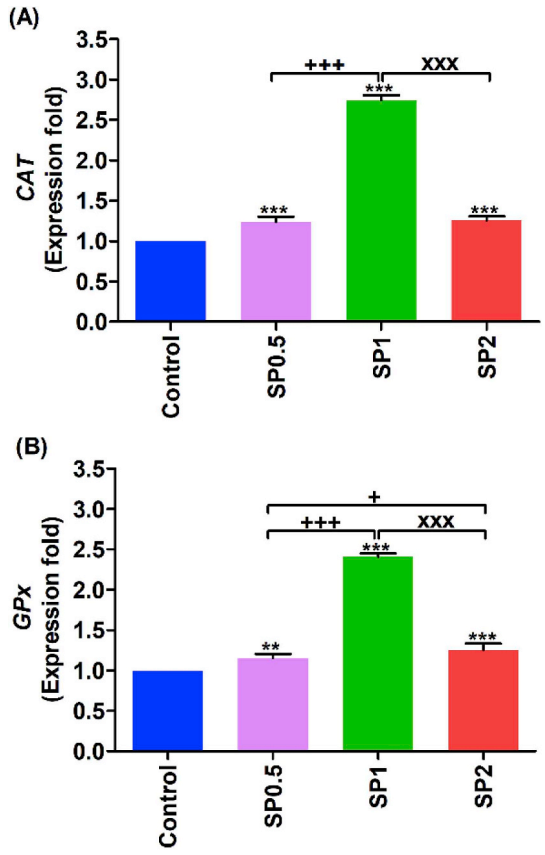

Fig. 4. RT-PCR validation of hepatic (A) *CAT* and (B) *GPx*. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control. $^{+}P < 0.05$ and $^{+++}P < 0.05$ vs. SP0.5. $^{xxx}P < 0.001$ vs. SP1. Statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test for multiple comparisons. SP0.5, fish group received basal diet containing 0.5% of SP. SP1, fish group received basal diet containing 1% of SP. SP2, fish group received basal diet containing 2% of SP.CAT, catalase. GPx, glutathione peroxidase.

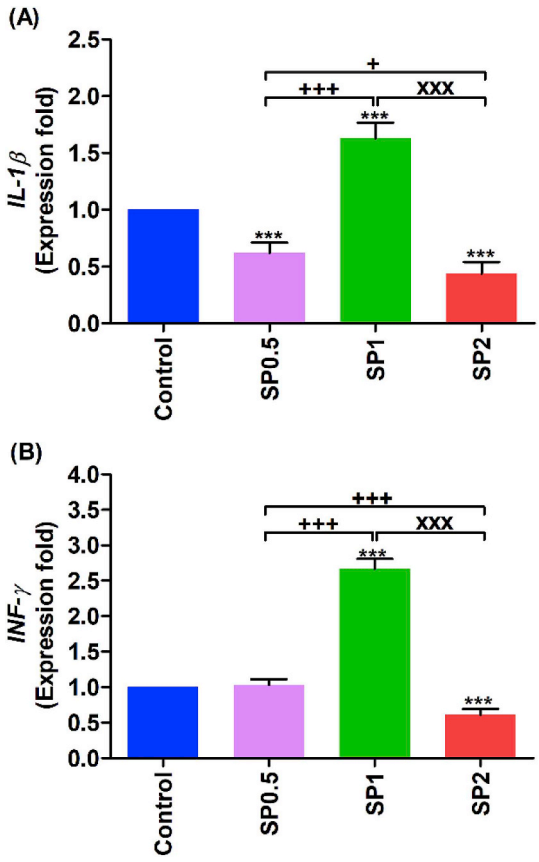

Fig. 5. RT-PCR validation of renal (A) *IL-1β* and (B) *INF-*γ. $^{***}P < 0.001$ vs. control. $^{+}P < 0.05$ and $^{+++}P < 0.05$ vs. SP0.5. $^{xxx}P < 0.001$ vs. SP1. Statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test for multiple comparisons. SP0.5, fish group received basal diet containing 0.5% of SP. SP1, fish group received basal diet containing 1% of SP. SP2, fish group received basal diet containing 2% of SP. *IL-1β*, interleukin-1 beta. *INF-*γ, interferon-gamma.

*IFN-*γ *is* a cytokine critical for innate and adaptive immunity against different infections. It is mainly secreted by Th1 and NK cells and regulates both innate and cell-mediated immune responses [51]. It drives macrophages to produce toxic agents that kill intracellular bacteria [52]. *IFN-*γ expressed constitutively in head kidney, skin, intestine, muscle, and brain [53].

5. Conclusion

SP supplemented diet had no effects on the growth performance of Nile tilapia, but improved the immune response via augmentation of the serum protein and globulin levels, enhancement of antioxidative indices, and upregulation of inflammatory cytokines, especially at a concentration 1% of the basal diet. From this result, the use of SP roots in fish farms is recommended to increase the immune response and could help to combat infectious diseases.

Conflicts of interest

The authors declare no conflict of interest.

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References

[1] H.A. Hassanien, J. Gilbey, Genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*) revealed by DNA microsatellites, Aquacult. Res. 36 (2005) 1450–1457, https://doi.org/10.1111/j.1365-2109.2005.01368.x.
[2] W. Wang, J. Sun, C. Liu, Z. Xue, Application of Immunostimulants in Aquaculture: Current Knowledge and Future Perspectives vol 48, (2016), pp. 1–23, https://doi.org/10.1111/are.13161.
[3] S. Gallage, T. Katagiri, M. Endo, M. Maita, Comprehensive evaluation of immunomodulation by moderate hypoxia in *S. agalactiae* vaccinated Nile tilapia, Fish Shellfish Immunol. 66 (2017) 445–454, https://doi.org/10.1016/j.fsi.2017.05.041.
[4] M. Moustafa, A.E. Eissa, A.M. Laila, A.Y. Gaafar, I. Abumourad, M. Elgendy, Investigations into the potential causes of mass kills in mari-cultured gilthead sea bream (*Sparus aurata*) at northern Egypt, Res. J. Pharmaceut. Biol. Chem. Sci. 6 (2015) 466–477.
[5] K. Grigorakis, G. Rigos, Aquaculture effects on environmental and public welfare – the case of Mediterranean mariculture, Chemosphere 85 (2011) 899–919, https://doi.org/10.1016/j.chemosphere.2011.07.015.
[6] S.-C. JI, O. Takaoka, G.-S. Jeong, S.-W. Lee, K. Ishimaru, M. Seoka, K. Takii, Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream Pagrus major, Fish. Sci. 73 (2007) 63–69, https://doi.org/10.1111/j.1444-2906.2007.01302.x.
[7] G. Immanuel, V. Vincybai, V. Sivaram, A. Palavesam, M. Marian, Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles, Aquaculture 236 (2004) 53–65, https://doi.org/10.1016/J.AQUACULTURE.2003.11.033.
[8] R. Chakrabarti, P.K. Srivastava, N. Verma, J. Sharma, Effect of seeds of *Achyranthes aspera* on the immune responses and expression of some immune-related genes in carp *Catla catla*, Fish Shellfish Immunol. 41 (2014) 64–69, https://doi.org/10.1016/j.fsi.2014.03.007.
[9] B. Vaseeharan, R. Thaya, Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture, Aquacult. Int. 22 (2014) 1079–1091, https://doi.org/10.1007/s10499-013-9729-3.
[10] A.E.-D.H. Sayed, Y.S. El-Sayed, A.H. El-Far, Hepatoprotective efficacy of *Spirulina*

platensis against lead-induced oxidative stress and genotoxicity in catfish; *Clarias gariepinus*, Ecotoxicol. Environ. Saf. 143 (2017) 344–350, https://doi.org/10.1016/j.ecoenv.2017.05.013.
[11] N. Gobi, B. Vaseeharan, R. Rekha, S. Vijayakumar, C. Faggio, Bioaccumulation, cytotoxicity and oxidative stress of the acute exposure selenium in *Oreochromis mossambicus*, Ecotoxicol. Environ. Saf. (2018), https://doi.org/10.1016/j.ecoenv.2018.06.070.
[12] A. Iswarya, B. Vaseeharan, M. Anjugam, N. Gobi, M. Divya, C. Faggio, β-1, 3 glucan binding protein based selenium nanowire enhances the immune status of *Cyprinus carpio* and protection against *Aeromonas hydrophila* infection, Fish Shellfish Immunol. (2018), https://doi.org/10.1016/j.fsi.2018.08.057.
[13] N. Gobi, B. Vaseeharan, J.C. Chen, R. Rekha, S. Vijayakumar, M. Anjugam, A. Iswarya, Dietary supplementation of probiotic *Bacillus licheniformis* Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*, Fish Shellfish Immunol. (2018), https://doi.org/10.1016/j.fsi.2017.12.066.
[14] H. Ahmad, Biological activities of *Salvadora persica* L. (Meswak), Med. Aromatic Plants 02 (2012) 1–5, https://doi.org/10.4172/2167-0412.1000129.
[15] A. Kumari, A.K. Parida, J. Rangani, A. Panda, Antioxidant activities, metabolic profiling, proximate analysis, mineral nutrient composition of *Salvadora persica* fruit unravel a potential functional food and a natural source of pharmaceuticals, Front. Pharmacol. 8 (2017) 61, https://doi.org/10.3389/fphar.2017.00061.
[16] M.A. Lebda, A.H. El-Far, A.E. Noreldin, Y.H.A. Elewa, S.K. Al Jaouni, S.A. Mousa, Protective effects of miswak (*Salvadora persica*) against experimentally induced gastric ulcers in rats, Oxid. Med. Cell. Longev. 2018 (2018) 1–14, https://doi.org/10.1155/2018/6703296.
[17] M. Abhary, A.-A. Al-Hazmi, Antibacterial activity of Miswak (*Salvadora persica* L.) extracts on oral hygiene, J. Taibah Univ. Sci. 10 (2016) 513–520, https://doi.org/10.1016/j.jtusci.2015.09.007.
[18] N. Alili, J.C. Türp, E.M. Kulik, T. Waltimo, Volatile compounds of *Salvadora persica* inhibit the growth of oral Candida species, Arch. Oral Biol. 59 (2014) 441–447, https://doi.org/10.1016/J.ARCHORALBIO.2014.02.001.
[19] H. Syawal, Syafriadiman, S. Hidayah, The use of miswak (*Salvadora persica* L.) extract to increase immune response of common carp (*Cyprinus carpio* L.) in cage, J. Biol. Divers. 9 (2008) 44–47.
[20] N.M.A. Olvera, P.C.A. Martinez, C.R. Galvan, S.C. Chavez, The use of seed of the leguminous plant *Sesbania grandiflora* as a partial replacement for fish meal in diets for tilapia (*Oreochromis mossambicus*), Aquaculture 71 (1988) 51–60, https://doi.org/10.1016/0044-8486(88)90272-4.
[21] G. Degani, S. Viola, D. Levanon, Effects of dietary carbohydrate source on growth and body composition of the European eel (*Anguilla anguilla* L.), Aquaculture 52 (1986) 97–104, https://doi.org/10.1016/0044-8486(86)90029-3.
[22] K. Jauncey, B. Ross, A Guide to tilapia Feeds and Feeding, Institute of Aquaculture, University of Stirling, Stirling., 1982.
[23] D.A. Higgs, J.R. McBride, J.R. Markert, B.S. Dosanjh, M.D. Plotnikoff, W.C. Clarke, Evaluation of Tower and Candle rapeseed (canola) meal and Bronowski rapeseed protein concentrate as protein supplements in practical dry diets for juvenile chinook salmon (*Oncorhynchus tshawytscha*), Aquaculture 29 (1982) 1–31, https://doi.org/10.1016/0044-8486(82)90030-8.
[24] E. Coles, Veterinary Clinical Pathology, fourth ed., W.B. Saunders, Philadelphia, 1986.
[25] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358, https://doi.org/10.1016/0003-2697(79)90738-3.
[26] E. Beutler, O. Durno, B.M. Kelly, Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61 (1963) 882–888.
[27] M. Nishikimi, N. Appaji Rao, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem. Biophys. Res. Commun. 46 (1972) 849–854, https://doi.org/10.1016/S0006-291X(72)80218-3.
[28] H. Aebi, Catalase *in vitro*, Methods Enzymol. 105 (1984) 121–126, https://doi.org/10.1016/S0076-6879(84)05016-3.
[29] D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70 (1967) 158–169.
[30] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione S-transferases, The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974) 7130–7139.
[31] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[32] J. Yuan, A. Reed, F. Chen, C.N. Stewart, D. Klein, S. Bustin, S. Mocellin, C. Rossi, P. Pilati, D. Nitti, F. Marincola, G. Mason, P. Provero, A. Vaira, G. Accotto, C. Heid, J. Stevens, K. Livak, P. Williams, U. Gibson, C. Heid, P. Williams, M. Pfaffl, M. Pfaffl, G. Horgan, L. Dempfle, K. Livak, T. Schmittgen, P. Cook, C. Fu, M. Hickey, E. Han, K. Miller, S. Zenoni, L. Reale, G. Tornielli, L. Lanfaloni, A. Porceddu, A. Ferrarini, C. Moretti, A. Zamboni, A. Speghini, F. Ferranti, M. Pezzotti, H. Eleaume, S. Jabbouri, H. Shen, L. He, T. Sasaki, Y. Yamamoto, S. Zheng, A. Ligaba, X. Yan, S. Ahn, M. Yamaguchi, S. Hideo, S. Matsumoto, M. Kutner, C. Nachtsheim, J. Neter, L. William, M. Hollander, D. Wolfe, S. Bustin, V. Benes, T. Nolan, M. Pfaffl, F. Pattyn, F. Speleman, A. De Paepe, J. Vandesompele, S. Peirson, J. Butler, R. Foster, W. Liu, D. Saint, A. Tichopad, M. Dilger, G. Schwarz, M. Pfaffl, R. Rasmussen, Y. Yang, J. Yuan, J. Ross, J. Noel, E. Pichersky, F. Chen, Statistical analysis of real-time PCR data, BMC Bioinf. 7 (2006) 85, https://doi.org/10.1186/1471-2105-7-85.
[33] M.A. Farag, S. Fahmy, M.A. Choucry, M.O. Wahdan, M.F. Elsebai, Metabolites profiling reveals for antimicrobial compositional differences and action mechanism in the toothbrushing stick "miswak" *Salvadora persica*, J. Pharm. Biomed. Anal. 133 (2017) 32–40, https://doi.org/10.1016/j.jpba.2016.11.018.
[34] K.H. El-Kholy, T.S.T. Seleem, T.A. El-Aasar, A. Hanaa Abdelharith, Effect of dietary addition of arak (*Salvadora persica*) on growth and reproductive performance in Black Baladi rabbit males, World Rabbit Sci. 16 (2010) 21–27, https://doi.org/10.4995/wrs.2008.639.
[35] A.K. Alm El-Dein, S.M.M. Mousa, S. Abd El-Aziz, Comparative study between miswak and neomycin on some productive, reproductive and immunological traits of Dokki4 chickens strain, Egypt, Poultry Sci. J. 34 (2014) 503–519, https://doi.org/10.21608/epsj.2014.5358.
[36] E. Yilmaz, S. Ergün, S. Ilmaz, Influence of carvacrol on the growth performance, hematological, non-specific immune and serum biochemistry parameters in rainbow trout (*Oncorhynchus mykiss*), Food Nutr. Sci. 06 (2015) 523–531, https://doi.org/10.4236/fns.2015.65054.
[37] G.F. Wiegertjes, R.J. Stet, H.K. Parmentier, W.B. van Muiswinkel, Immunogenetics of disease resistance in fish: a comparative approach, Dev. Comp. Immunol. 20 (1996) 365–381.
[38] A.H. El Rabey, M.F. Almutairi, I.A. Al Sieni, N.M. Al Seeni, A.M. Al Duais, I.M. Sakran, O.A. Abuelgassim, *Salvadora persica* leaf aqueous extract attenuates hyperglycemia and hyperlipidemia in alloxan induced diabetic male rats, Biomed. Res. 29 (2018) 2424–2434, https://doi.org/10.4066/biomedicalresearch.64-18-549.
[39] K.S. Ramadan, S.A. Alshamrani, Effects of *Salvadora persica* extract on the hematological and biochemical alterations against immobilization-induced rats, Sci. Tech. Rep. 2015 (2015) 1–5, https://doi.org/10.1155/2015/253195.
[40] M. Khan, M. Ali, A. Ali, S.R. Mir, Hypoglycemic and hypolipidemic activities of Arabic and Indian origin *Salvadora persica* root extract on diabetic rats with histopathology of their pancreas, Int. J. Health Sci. 8 (2014) 45–56.
[41] E. Noumi, M. b Snoussi, N. Trabelsi, H. Hajlaoui, R. Ksouri, E. Valentin, A. Bakhrouf, Antibacterial, anticandidal and antioxidant activities of *Salvadora persica* and *Juglans regia* L. extracts, J. Med. Plants Res. 5 (2011) 4138–4146.
[42] B. Al-Dabbagh, I.A. Elhaty, C. Murali, A. Al Madhoon, A. Amin, *Salvadora persica* (miswak): antioxidant and promising antiangiogenic insights, Am. J. Plant Sci. 09 (2018) 1228–1244, https://doi.org/10.4236/ajps.2018.96091.
[43] M.M. Ibrahim, A.A.A. AL Sahli, I.A. Alaraidh, A.A. Al-Homaidan, E.M. Mostafa, G.A. EL-Gaaly, Assessment of antioxidant activities in roots of Miswak (*Salvadora persica*) plants grown at two different locations in Saudi Arabia, Saudi J. Biol. Sci. 22 (2015) 168–175, https://doi.org/10.1016/j.sjbs.2014.11.019.
[44] T. Ahmed, S. Abdullah, K. Abbas, M.A. Zia, Catalase enzyme response to chronic Pb +Cd metal mixture exposure, its purification and partial characterization from the kidney of freshwater fish, *Oreochromis niloticus*, Pakistan J. Zool. 48 (2016) 1733–1740, https://doi.org/10.21162/PAKJAS/16.3256.
[45] O.Y. Vasylkiv, O.I. Kubrak, K.B. Storey, V.I. Lushchak, Catalase activity as a potential vital biomarker of fish intoxication by the herbicide aminotriazole, Pestic. Biochem. Physiol. 101 (2011) 1–5, https://doi.org/10.1016/j.pestbp.2011.05.005.
[46] D.A. Monteiro, H.S. Selistre-de-Araújo, D. Tavares, M.N. Fernandes, A.L. Kalinin, F.T. Rantin, -C. Alternagin-C (ALT, A disintegrin-like cys-rich protein isolated from the venom of the snake *Rhinocerophis alternatus*, stimulates angiogenesis and antioxidant defenses in the liver of freshwater fish, *Hoplias malabaricus*, Toxins 9 (2017), https://doi.org/10.3390/toxins9100307.
[47] C. Rocha-Santos, F.F. Bastos, R.F. Dantas, R.A. Hauser-Davis, L.C. Rodrigues, V.L.F. Cunha Bastos, J. Cunha Bastos, Glutathione peroxidase and glutathione S-transferase in blood and liver from a hypoxia-tolerant fish under oxygen deprivation, Ecotoxicol, Environ. Saf. 163 (2018) 604–611, https://doi.org/10.1016/J.ECOENV.2018.06.089.
[48] P.H.V. Saavedra, D. Demon, H. Van Gorp, M. Lamkanfi, Protective and detrimental roles of inflammasomes in disease, Semin. Immunopathol. 37 (2015) 313–322, https://doi.org/10.1007/s00281-015-0485-5.
[49] C.N. LaRock, B.T. Cookson, Burning down the house: cellular actions during pyroptosis, PLoS Pathog. 9 (2013) e1003793, , https://doi.org/10.1371/journal.ppat.1003793.
[50] H. Bessler, H. Salman, M. Bergman, Y. Alcalay, M. Djaldetti, *In vitro* effect of lycopene on cytokine production by human peripheral blood mononuclear cells, immunol, Invest 37 (2008) 183–190, https://doi.org/10.1080/08820130801967809.
[51] J.R. Schoenborn, C.B. Wilson, Regulation of interferon-γ during innate and adaptive immune responses, Adv. Immunol. 96 (2007) 41–101, https://doi.org/10.1016/S0065-2776(07)96002-2.
[52] K. Schroder, P.J. Hertzog, T. Ravasi, D.A. Hume, Interferon-γ: an overview of signals, mechanisms and functions, J. Leukoc. Biol. 75 (2004) 163–189, https://doi.org/10.1189/jlb.0603252.
[53] J. Velázquez, J. Acosta, N. Herrera, A. Morales, O. González, F. Herrera, M.P. Estrada, Y. Carpio, Novel IFNγ homologue identified in Nile tilapia (*Oreochromis niloticus*) links with immune response in gills under different stimuli, Fish Shellfish Immunol. 71 (2017) 275–285, https://doi.org/10.1016/j.fsi.2017.10.014.
[54] NRC (Ed.), Nutrient Requirements of Poultry, National Academies Press, Washington, D.C., 1994, , https://doi.org/10.17226/2114.