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Enhancement of Antioxidant Activity, Non-specific Immunity and Growth Performance of Nile Tilapia, *Oreochromis Niloticus* by Dietary Fructooligosaccharide

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Abstract

In this study, Nile tilapia was fed experimental diets containing different levels (0, 1, 2, and 3%) of fructooligosaccharide (FOS) for 6 weeks to investigate its effect on the antioxidant activity, non-specific immunity and growth performance of Nile tilapia. Liver and serum samples were taken after 3 and 6 weeks feeding. Results showed that malondialdehyde level and superoxide dismutase activity decreased significantly ($P<0.05$) with dietary FOS supplementation after 3 and 6 weeks feeding compared to the control. Catalase and glutathione peroxidase activities were significantly decreased in groups fed 1 and 2% FOS for 3 weeks. Serum immunoglobulin M and lysozyme activity were significantly increased with dietary FOS after 3 and 6 weeks feeding. Nitric oxide revealed significant increase with 2% dietary FOS after 3 weeks feeding and there were no significant difference ($P>0.05$) in other treated groups fed for 6 weeks compared to the control. Weight gain also recorded significant increase in group fed 2% FOS for 6 weeks. These results indicated that dietary FOS supplementation could significantly enhance the antioxidant activities, non-specific immune response and growth performance of *Oreochromis niloticus*. It could be conclude that 2% dietary FOS was the most suitable and beneficial dose for Nile tilapia.

Keywords: Antioxidant enzymes activity; Growth performance; Fructooligosaccharide; Nile tilapia; Non-specific immunity

Introduction

Nile tilapia, *Oreochromis niloticus (O. niloticus)* is considered as one of the most important freshwater species for commercial aquaculture in Egypt, due to its high nutritional values, rapid growth rate and resistance to diseases [1]. However, bacterial diseases outbreak continues to occur among cultured *O. niloticus* due to high intensification, causing considerable economic losses in fish farms. Several approaches such as vaccination and chemotherapy have been carried out to increase fish immunocompetence and prevent aquatic diseases [2,3].

On the other hand, there are strict regulations on the application of antibiotics and chemotherapeutics in aquaculture because of its negative impacts which includes the development of antibiotic resistant bacteria, suppression of host's immune system, destruction of the microbial population in the aquatic environment and bioaccumulation [4,5]. Therefore, increasing attention is being paid to the dietary supplementation of an alternative friendly probiotics, prebiotics and immunostimulants which help to improve fish immune response and hence reduce the susceptibility of fish to diseases [6-8].

Prebiotics are defined as non-digestible feed ingredients that stimulating the growth and metabolism of beneficial bacteria in the host gastrointestinal tract [9]. Fructooligosaccharide (FOS or oligofructose) is one of the most common prebiotics used in human, terrestrial animals and has received great attention as dietary supplement for different finfish species during the past years [10-13] as well as shellfish [14]. Beneficial effects of dietary FOS supplementation on growth performance, immune response and disease resistance in several fish species have been demonstrated in previous studies [12,15-19].

Furthermore, FOS has shown positive effects on the fish antioxidant activity [12,13,20,21]. However, other studies have reported that FOS has no beneficial effects on fish growth or immunity [7,10,22-24] or even has adverse effect on fish performance [25]. Therefore, prebiotics evaluations are recommended before suggesting specific prebiotic strategies for certain fish species [26]. The aim of the present study was to evaluate the effect of different dietary levels of FOS on the antioxidant capability, non-specific immunity and growth performance of Nile tilapia.

Materials and Methods

Experimental diets and fish

Fructoligosaccharides powder (FOS) (Nutraflora)® obtained from GTC Nutrition company (Westchester, USA) was added at a different concentrations on the commercial basal diet (Joe Trade Company, Egypt) containing 30% crude protein. The first diet was kept as a control without additive. The second, third and fourth diets were contained FOS at a concentration of 1, 2% and 3% (w/w) respectively. The experimental diets were repellted using a hand pelletizer with a die of 3 mm and left air-dried at room temperature. After drying, the diets were broken up into appropriate size and stored in tight plastic bags at 4°C. The proximate analysis of basal diet was carried out according to the AOAC standard [27]. Ingredients and proximate analysis of the basal diet and Gross energy was calculated by Brett [28] in Table 1.

Ingredients	(%)	Proximate analysis	%
Fish meal	15	Dry matter	89.07
Yellow Corn meal	28	Crude protein	30
Soybean meal	41	Crude fat	3.17
Wheat bran meal	10	Ash	10.76
Corn oil	4	Crude fiber	4.94
Vitamin mix a Mineral mix b	1 1	Nitrogen free extract (NFE)c	40.2
Total	100	Gross energy (MJ/Kg)d	15.24
[a]Vitamin (each 3 kg) supply the following: vitamin A, 1200.000 IU; vitamin D3, 300.000 IU; Vitamin E, 700 mg; vitamin K3, 500 mg; vitamin B1, 500 mg; vitamin B2, 200 mg; vitamin B6, 600 mg; vitamin B12, 3 mg; ascorbic acid, 450 mg; Biotin, 6 mg; panthonic acid, 670 mg; methionine, 3.000 mg; folic acid, 300 mg; chlolin chloride, 10.000 mg.			
[b]Mineral (each kg) contain the following: zinc sulphate, 50.000 mg; copper, 4.000 mg; ferrous sulphate, 20.000 mg; Manganous sulphate, 60.000 mg; sodium, 2.000 mg; selenium, 100 mg; cobalt chloride, 100 mg; iodine, 500 mg; c NFE = 100 - (% crude protein + % crude fat + % ash + % crude fiber + % moisture); d: Gross energy was calculated according to Brett [28].			

Table 1: Ingredients and proximate analysis of basal diet.

Nile tilapia was obtained from private fish farm at Kafr El Sheikh Governorate, Egypt. The fish were transported to the Lab of Fish Diseases and Management at Fac. of Vet. Med, Benha University, Egypt and stocked in fiberglass tanks (750 L capacity). Fish were randomly distributed into eight tanks (35 fish per tank) and allowed to acclimate to lab conditions for 2 weeks before starting the feeding experiment. Each treatment was carried out in duplicate. During the acclimation period, fish were fed basal diet two times a day.

Experimental design

After acclimation, the first control group was fed on free FOS diet. The second, third and fourth groups have been received diets containing 1%, 2%, and 3% FOS respectively. All experimental groups were fed by hand, twice daily at 08:00 and 16:00 at a rate of 3% of their body weight for 6 weeks. Nearly half of tank water was exchanged every day to maintain water quality. The water quality parameters were determined according to the guidelines of APHA [29]. The water temperature was adjusted at 28 ± 2°C using electrical heaters (Xilong, China), pH was 7.1 ± 0.2 and the dissolved oxygen was maintained at 5.2 ± 0.7 mg L^{-1} along the experimental period. Fish were routinely checked for health and any mortality throughout the experiment.

Sampling

Two sampling points were taken after 3 and 6 weeks feeding. Three fish per tank (Total = 6 fish per treatment) were taken randomly and anaesthetized with benzocaine (80 mg L^{-1}). The blood was collected without anticoagulant from the caudal vessels of each individual fish using 3 ml sterile syringe (23-gauge needle) and allowed to clot at room temperature for 2 h. After that, all the samples were centrifuged at 3,000 rpm for 15 min at 4°C. The separated sera were pooled together (n=3) and stored at -80°C for later analysis of immunological parameters. Immediately after blood collection, the liver was quickly removed, weighted and stored at -80°C for lipid peroxidation (LPO) and antioxidant enzymes assay.

Lipid peroxidation and antioxidant enzymes assay

The liver was homogenized in cooled phosphate buffer saline pH 7.2 at a ratio 1:10 (w/v) using electrical homogenizer (Heidolph, Germany). The procedure was performed on ice. The homogenates were centrifuged at 13,000 rpm at 4°C for 15 min and the resultant supernatants were separated in aliquots and stored at -80°C until the determination of malondialdehyde (MDA) level and antioxidant enzymes which performed within one week after extraction. The liver malondialdehyde (MDA), which used as marker of LPO was measured according to Ohkawa et al. [30]. Superoxide dismutase (SOD) activity was determined using commercial kits (Biodiagnostic Company, Egypt) following the method described by Nishikimi et al. [31]. Catalase (CAT) activity was determined according to Sinha [32] and Glutathion peroxidase (GPX) activity was assayed according to Paglia and Valentine [33].

Immunological parameters assay

Serum lysozyme activity was measured using lysoplate assay [34]. Serum Immunoglobulin M (IgM) was measured spectrophotometrically following the manufacture protocol of Enzyme-Linked Immunosorbent Assay (ELISA) kits obtained from (Cusabio Biotech Co. Ltd, USA). Nitric oxide was determined using Griess reagent according to the method described by Granger et al. [35].

Growth performance

At the end of the experiment, growth performance and physiological indices were assessed according to the following formula: Weight gain (WG) = W2-W1, Feed conversion ratio (FCR) = F/WG; Condition (CF) factor = 100 × (body weight (g)/body length $(cm)^3$; Spleensomatic index (SSI) = weight of spleen (g)/total body weight (g)) ×100; Hepatosomatic Index (HSI) = weight of liver (g)/total body weight (g)) ×100. Where, W1 is initial weight (g), W2 is final weight (g), and F is amount of feed intake (g).

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test within each sampling time to determine the significant difference ($P<0.05$) between means using SPSS version 16.0 software package. All data were presented as means ± SE (standard error). Normality and homogeneity of variance for all obtained data were confirmed with Shapiro–Wilk and Levene's test respectively. Two-way analysis was carried out to evaluate the interaction between dietary FOS and feeding duration.

Results

Liver MDA content and SOD activity of Nile tilapia fed diets containing different levels of FOS were significantly ($P<0.05$) decreased after 3 and 6 weeks feeding compared to the control (Figures 1 and 2). The lowest SOD activity was observed in group fed 3% (361.67 ± 2.03 U/g) for 6 weeks compared to the control (460.67 ± 3.18 U/g). CAT activity exhibited significant decrease in groups fed 1 and 2% FOS for 3 weeks (14.0 ± 0.83 U/g) and (16.20 ± 0.97 U/g) respectively compared to the control (36.69 ± 2.03 U/g). However, after

6 weeks feeding, CAT activity recorded no significant difference with 2 and 3% dietary FOS (Figure 3). Moreover, GPX activity revealed significant decrease in group fed 1 and 2% dietary FOS for 3 weeks (201.0 ± 2.31 and 341.0 ± 5.77 U/g) respectively. While, after 6 weeks feeding, GPX activity exhibited significant decrease in all FOS treated groups (Figure 4). There were highly significant (P<0.001) interaction between dietary FOS and feeding duration on liver SOD and GPX activities.

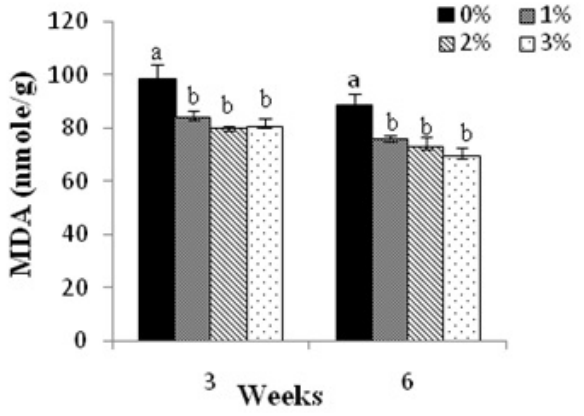

Figure 1: Lipid peroxidation as malondialdehyde (MDA) level in liver of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

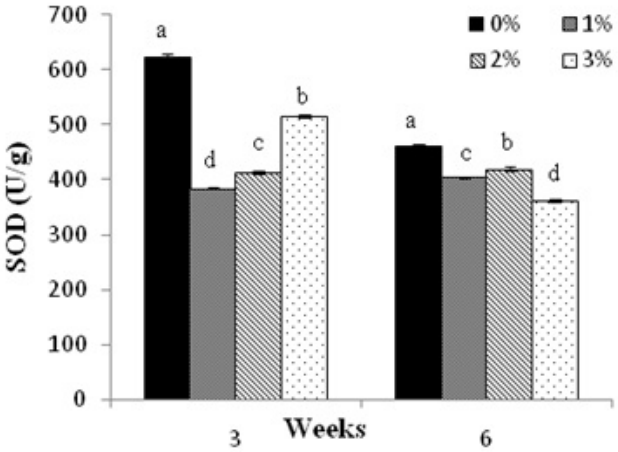

Figure 2: Superoxide dismutase (SOD) activity in liver of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

Serum lysozyme activity and IgM were significantly improved (P<0.05) with dietary FOS either after 3 or 6 weeks feeding compared to the control (Figures 5 and 6). It was observed that after 6 weeks feeding, fish received diet containing 3% FOS showed significant decrease in serum IgM (117.61 ± 2.62 µg/ml) compared to the group fed 1 and 2 % FOS (133.36 ± 2.75 and 129.89 ± 2.39 µg/ml) respectively. Nitric oxide was significantly (P<0.05) increased in group fed with 2% dietary FOS (24.15 ± 3.36 µmole/l) for 3 weeks feeding compared to the control (5.86 ±1.42 µmole/l). However, after 6 weeks, nitric oxide has not significantly affected in all treated groups in compare with the control (Figure 7). Interaction between dietary FOS and feeding duration was significantly (P<0.01) observed on serum nitric oxide level.

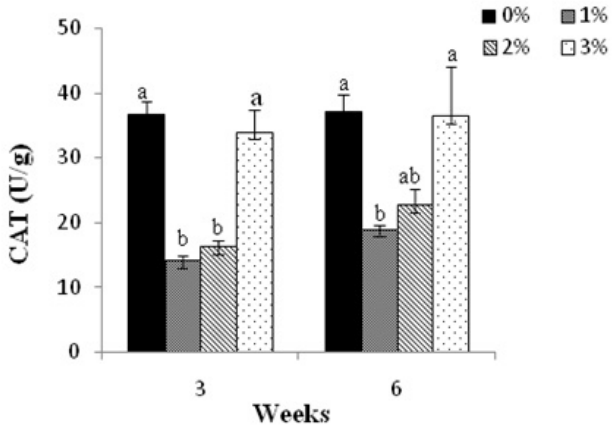

Figure 3: Catalase (CAT) activity in liver of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

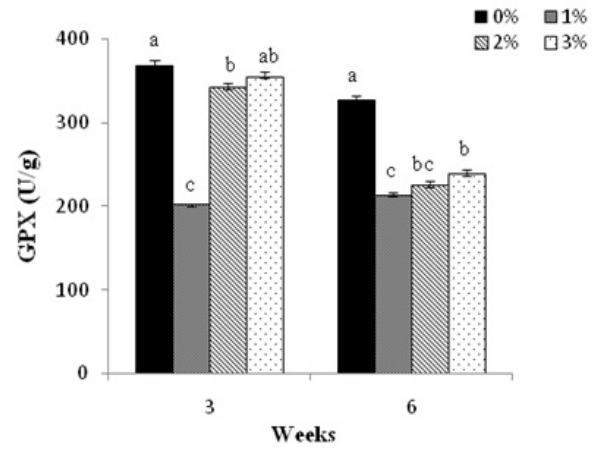

Figure 4: Glutathione peroxidase (GPx) activity in liver of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

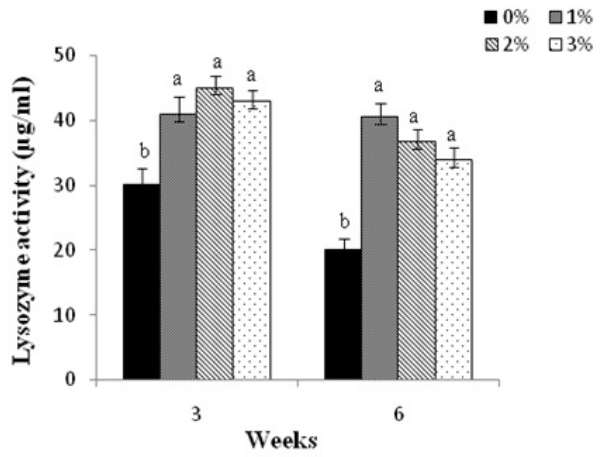

Figure 5: Serum lysozyme activity of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

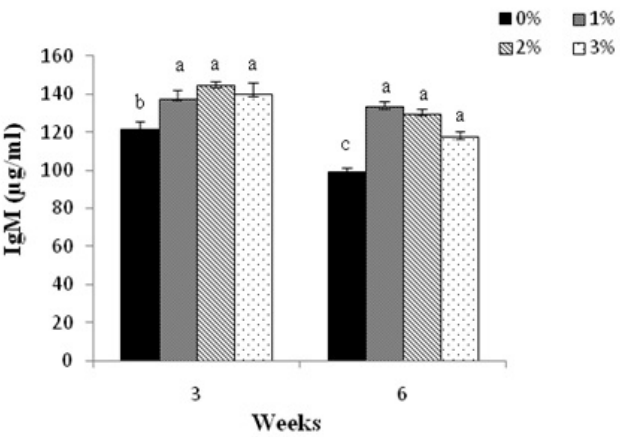

Figure 6: Serum immunoglobulin M (IgM) level of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

The effect of dietary FOS levels on the growth performance of Nile tilapia after 6 weeks feeding was shown in Table 2. Dietary supplementation of FOS had no significant effect (P>0.05) on growth performance and health status of Nile tilapia. However, weight gain was significantly increased in group supplemented with 2% dietary FOS compared to the other treatment groups. There were no mortalities among all treated groups throughout the experiment.

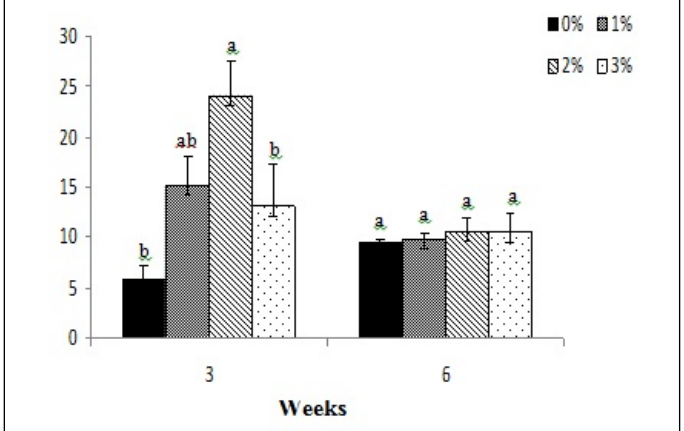

Figure 7: Nitric oxide (NO) activity (µ mol/l) in serum of Nile tilapia fed different levels of dietary FOS. Data represent the mean ± S.E. Values with different superscript letters are significantly different among group at each sampling point.

Parameters	0 %	1 %	2 %	3 %
Initial weight (g)	25.22 ± 2.19	25.22 ± 1.7	23.52 ± 2.15	24.4 ± 0.46
Final weight (g)	40.95 ± 3.35	45.2 ± 1.71	46.3 ± 1.21	43.51 ± 1.83
Weight gain (g)	15.74 ± 2.14^{b}	19.98 ± 0.99^{ab}	22.78 ± 2.68^{a}	19.11 ± 2.18^{ab}
FCR	2.18 ± 0.28	1.62 ± 0.15	1.49 ± 0.34	1.73 ± 0.21
CF (%)	1.43 ± 0.04	1.53 ± 0.05	1.5 ± 0.01	1.53 ± 0.03
HSI (%)	2.24 ± 0.25	2.31 ± 0.18	1.78 ± 0.39	2.32 ± 0.15
SSI (%)	0.39 ± 0.1	0.29 ± 0.04	0.25 ± 0.04	0.27 ± 0.03

Mean values (± S.E) in the same raw with different superscript letters are statistically different at (P<0.05) using one-way analysis of variance (ANOVA) and Duncan's multiple range test.

Table 2: Growth performance of *O. niloticus* fed different dietary levels of FOS for 6 weeks.

Discussion

Dietary supplement of prebiotics have shown great interest in aquaculture due to its growth performance enhancement and increasing resistance of fish to pathogens and environmental stressors through stimulating the host's immune response [8,36].

Malondialdehyde (MDA) is formed as an end product of lipid peroxidation, which is the initial step of cellular membrane damage caused by reactive oxygen species (ROS) [37]. In the present study, dietary supplementation of FOS significantly reduced liver MDA content after 3 and 6 weeks feeding. The decreased MDA indicated that FOS could inhibit the process of lipid peroxidation which is considered a biomarker of oxidative damage caused by free radicals [38]. A Similar result was observed in blunt snout bream, *Megalobrama amblycephala*

fed 0.4% FOS for 8 weeks [12]. On the other hand, LPO level was not affected by dietary FOS in turbot, *Scophthalmus maximus* [13].

Antioxidant enzymes SOD, CAT and GPX are considered the first line of antioxidant defense and served as sensitive biomarkers of oxidative stress [39]. SOD is considered the first enzyme responsible for scavenging ROS and protecting cells from damage by free radicals process [40]. The present results showed that liver SOD activity significantly decreased in Nile tilapia fed with different dietary FOS levels along the experimental period (6 weeks). This indicated that FOS possesses antioxidant potentials that might effectively reduce ROS production and its adverse effects. This explanation was supported by the fact that the higher SOD activity, the more superoxide radicals need to be reacted [41]. Guerreiro et al. [13] also recorded significant decrease in SOD activity of turbot, *Scophthalmus maximus* fed 0.5 and 1% short chain fructooligosaccharides for 9 weeks. However, liver SOD activity was significantly increased by dietary FOS for 8 weeks in other studies [6,12,21]. This difference might be attributed to fish species, prebiotic concentrations, feeding duration and feeding modes. CAT and GPX activity in the current study revealed significant decrease in Nile tilapia fed FOS at a concentration of 1 and 2% for 3 weeks, indicating that FOS has capability to reduce peroxide radicals and converting it into oxygen and water [42]. There was no significance difference in CAT activity with 2 and 3 % dietary FOS for 6 weeks compared to the control. This result could be explained that the exogenous antioxidants supplementation could reduce the gene expression of CAT enzyme and consequently decreases its activity [43]. Similar no significant effect on CAT activity has also been reported in blunt snout bream [12] and triangular bream *Megalobrama terminalis* [6]. The significant decrease of antioxidant enzymes activity in *O. niloticus* indicated that dietary FOS could maintain the redox state in the cell and minimize the adverse effects of ROS [44].

Several parameters such as respiratory burst activity, nitric oxide synthase, lysozyme activity, bactericidal activity, immunoglobulin level, antibody response, etc. are served as a good immunological indicator of fish health status [45]. In the present study, dietary FOS showed beneficial effect on the non-specific immune response of Nile tilapia evidenced by the significant increase of serum lysozyme activity and IgM level. The higher lysozyme activity probable attributed to the high leukocyte production with dietary FOS [6] due to the fact that fish lysozyme is mainly produced by neutrophiles and macrophages [46]. The immunostimulatory effect of FOS could be ascribed to the growth stimulation of beneficial bacteria such as lactobacilli and befidobacteria, which possess lipopolysaccharides that have immunostimulatory properties [47]. Moreover, acetate, propionate and lactic acid as end products of FOS fermentation play a crucial role in modulating the immune system [48]. Furthermore, FOS could interact with toll like receptors (TLR2) expressed on macrophages [49] and up-regulated the expression of antimicrobial peptides (Leap) which have important role in innate immune defense and hence disease resistance of fish [12]. Parallel to this study in a previous investigations, enhanced lysozyme activity has been recorded in red drum [26], Caspian roach fry [16], turbot [13] and blunt snout bream [12,21]. However, dietary FOS showed no significant effect on lysozyme activity in other studies [10,22]. This contradictory may be attributable to the prebiotic dosage, life stage and/or fish species [50].

Nitric oxide had also been shown to be a very important molecule in regulating immune functions as well as a direct antimicrobial effect [51]. In the current study, nitric oxide was significantly increased in *O. niloticus* fed 2% dietary FOS for 3 weeks; probably due to the increase of macrophage production by dietary FOS. Meanwhile, after 6 weeks feeding, serum nitric oxide showed no significant improvement in all treated groups compared to the control fed control diet. This result could be supported by Yoshida et al. [52] who reported that in African catfish fed 10 g kg^{-1} prebiotic mannanoligosaccharide, the number of activated neutrophils increased during the first 2 weeks and then decreased back to the control level after 45 days.

In the present study, growth performance of *O. niloticus* fed FOS showed improvement but not statistically differ. Weight gain in group fed 2% dietary FOS for 6 weeks revealed significant increase compared to the control fed basal diet. The improved weight gain could be attributed to the enhanced digestive enzymes activity and microvilli height with dietary FOS supplementation [12,19] or stimulation the growth of beneficial probiotic Bacillus spp. [53]. Growth improvement by dietary FOS has previously been reported in different fish species [12,16,17,54]. On the other hand, lack of growth response to dietary FOS was observed in other studies [7,15,22-24]. The discrepancy of growth response to dietary FOS seems to differ depending on species, fish size, different intestinal moropholgy, gut microbiota, FOS concentration and feeding duration [50]. Furthermore, negative effects of dietary prebiotics on fish performance have been recorded in beluga sturgeon [25] and Arctic charr [55]. The negative impacts of dietary prebiotics have been attributed to inability of intestinal microbiota to ferment excessive levels of prebiotics and subsequent accumulated in the intestine which might have deleterious effect to the enterocytes [25].

Condition factor (CF) and organ indices such as hepatosomatic (HSI) index, and spleenosomatic index (SSI) could be used as indicators of the health status of fish [56]. In the present work, there was no significant effect on CF, HSI and SSI of *O. niloticus* fed FOS indicating that FOS supplement had no detrimental effects on liver tissue or general fish health. This agreed with results of other studies [7,10,16,54]. These results supported by the assumption that increase of these indices such as hepatosomatic index could be resulted from increased production of endoplasmic reticulum for protein synthesis in the liver tissue or hypertrophy and hyperplasia of liver cell in fish exposed to stress [57].

The present study concluded that dietary FOS supplementation had an enhancement effects on non-specific immunity and growth performance of Nile tilapia as well as it protect cells against the adverse effects of ROS. Dietary supplementation with 2% FOS for 6 weeks is considered the most suitable dose for Nile tilapia to be involved in aquafeed to improve aquaculture industry.

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