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## Impact of Sel-Plex® dietary supplementation on growth performance, physiological response, oxidative status, and immunity-linked gene expression in Nile tilapia (*Oreochromis niloticus*) fingerlings challenged with *Aeromonas hydrophila*

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### Abstract

**Background:** Organic selenium (Sel-Plex®) supplementation holds considerable promise for improving the effectiveness of fish production.

**Aim:** This experiment was accomplished to judge the potential benefits of Sel-Plex® nutritional additive on growth outcomes, physiological response, oxidative status, and immunity-linked gene expression in Nile tilapia (*Oreochromis niloticus*) fingerlings exposed to bacterial infection with *Aeromonas hydrophila*.

**Methods:** Utilizing a basal diet of 30% protein, four experimental diets were prepared, each of which contained Sel-Plex® at concentrations of 0.0, 0.5, 1, and 2 mg/kg, respectively. Three replicates of 20 fish/treatment were used using 240 healthy Nile tilapia fingerlings. Fish were placed in 12 glass aquariums and separated into 4 groups at random. For the entire span of 8 weeks, diets were admitted to fish at a 3% rate of fish biomass/aquarium. After the feeding trial, pathogenic *A. hydrophila* was intraperitoneally injected into fish of each treatment, and fish were observed for 15 days to track the survival rate (SR) after the challenge.

**Results:** Growth performance, physiological response, immunological parameters (phagocytic activity, phagocytic index, and lysozyme), and antioxidant parameters [catalase, superoxide dismutase (SOD), malondialdehyde, and glutathione peroxidase (GPx)] were noticeably improved in Sel-Plex® treated groups. Moreover, Sel-Plex® increased gene expression linked with the immune system in the liver (tumor necrosis factor- $\alpha$  and interleukin 1 $\beta$ ), to growth (insulin-like growth factor 1 and growth hormone receptor), and antioxidants (SOD and GPx). Under pathogen-challenge conditions, the employed dietary Sel-Plex® supplementation could successfully lower fish oxidative stress, offering a potential preventive additive for Nile tilapia instead of antibiotics. On the other hand, Sel-Plex® significantly enhanced each of three intestinal morphological measurements (villus width, villus length, and crypt depth), demonstrating the greatest influence on the improvement of intestinal structure overall. In the Nile tilapia control group, the infection with *A. hydrophila* caused noticeable degenerative alterations in the gut, hepatopancreas, spleen, and posterior kidney. The severity of the lesion was significantly reduced and significantly improved with higher Sel-Plex® concentrations. Sel-Plex® supplemented groups had 100% SRs among the *A. hydrophila*-challenged groups.

**Conclusion:** It could be advised to enrich the diets of Nile tilapia fingerlings with 1–2 mg.kg<sup>-1</sup> of Sel-Plex® to enhance growth rate, physiological response, immunological reaction, and intestinal absorptive capacity

**Keywords:** *Aeromonas hydrophila*, Immune-related gene expression, Nile tilapia, Organic selenium, Oxidative status.

### Introduction

Aquaculture and fisheries constitute a crucial part of the world's production of food, health, and nutrient-rich food suppliers (Waite *et al.*, 2014). Seafood helps

people consume critical nutrients such as vitamin B, selenium, iodine, zinc, calcium, phosphorus, copper, and iron as well as the fat-soluble D, and A vitamins. It is an effective protein and energy source possessing

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a significant nutritive value (Jayasekara *et al.*, 2020). In comparison to fast food and manufactured meat byproducts, fish and shellfish products seem to be the healthier option among animal products (Boukid *et al.*, 2022). Consumption of fish is typically recognized as an element of appropriate dietary practices since it is a plentiful supply of essential elements, such as vitamins (A and D3), highly digestible proteins, trace minerals (selenium and iodine), and n-3 long-chain polyunsaturated fatty acids. In aquaculture, exogenous feeding provides the opportunity to modify the nutritional makeup of the fish (Ribeiro *et al.*, 2019).

Nile tilapia (*Oreochromis niloticus*) is a highly valuable freshwater species for commercial aquaculture in Egypt; that may be attributed to its quick development rate, high nutritional value, and disease resilience. However, the latest trend in Egyptian tilapia farming is the direction of further intensification of culture systems, which frequently causes stressful conditions and depressingly disturbs their development and welfare and makes fish more susceptible to stress, and other diseases caused by stress (Dawood *et al.*, 2019). Fish feed is mostly expensive input in aquaculture operations since fish nutrition is a crucial factor in managing the health of farmed finfish and shellfish (Kaur *et al.*, 2017). Fortification is the deliberate addition of one or more micronutrients (such as vitamins and minerals) to a food or condiment to be able to enhance the dietary effect of the food supply and benefit the general public health with little risk to the health. Micronutrients can assist in replacing the micronutrient content lost during processing in addition to enhancing the nutritional value of staple meals (Nölle *et al.*, 2020).

As aquaculture production frameworks are expanded, fish are subjected to an increasing number of ecological conditions, such as overcrowding, low water quality, improper handling, and shipping (Sakai, 1999). These stressors might have adverse effects on fish health and development, and they might also make aquaculture systems less effective. The majority of feed supplements used in animal diets including; immunostimulants, antimicrobials, antioxidants, and herbal plants, increase the development performance profitability, survival, and disease resilience of Nile tilapia (*O. niloticus*); lowering the susceptibility to diseases and protecting fish from stress and diseases especially in winter (Abdelkhalek *et al.*, 2015).

Dietary carbohydrates known as prebiotics cannot be digested and do not go through the upper gastrointestinal tract. Prebiotics have a positive effect on the host by selectively encouraging the proliferation and/or stimulating the metabolic activity of beneficial bacteria in the alimentary tract (Manning and Gibson, 2004). Another way they change the composition of gut bacteria is by modifying the substrate type that is available to the current intestinal microbiota (Mei *et al.*, 2011).

Aquaculture producers have developed certain technologies that support quick developmental performance through growth promoters and feed additives because this industry is committed to attaining the lowest feed conversion ratio (FCR) and fastest growth (Van Doan *et al.*, 2019). Viruses, parasites, and bacteria are the main disease causes in the aquaculture industry nowadays (Rigos *et al.*, 2021). The largest issue confronting the aquaculture sector is how to maintain fish immune competence and disease resistance while dealing with increased feed costs and treatment limitations (Dawood *et al.*, 2018a).

Tilapia has a stronger need for selenium and needs higher dosages of such mineral in their meals to have an impact on their development characteristics. These effects may depend on the chemical composition of the introduced selenium, the fish development stage, or the timing of the treatment (Durigon *et al.*, 2019). Selenium is a vital dietary component that aids in immune system health, promotes a normal cellular immunological response, and strengthens the body's defenses against infection with viral (Rayman, 2000). According to Sunde (2006), selenium can be found in two different forms: organic (selenocysteine and selenomethionine) and bioavailable inorganic (selenite and selenate). Because the two selenium forms are so similar, the organic selenium (selenomethionine) is taken up as an amino acid by the intestines using comparable procedures to methionine. On the other hand, inorganic selenium is passively absorbed (Shini *et al.*, 2015). Selenium shortage in aquaculture diets can have a number of negative consequences, including slow development, lower performance, weakened immunity, and illnesses that can result in high financial defeats (Naiel *et al.*, 2022).

Dietary selenium effectiveness depends on its type. This has caused an industry-wide movement away from the conventional inorganic form of sodium selenite and toward safer, organic forms of selenium. Because of its high toxicity, pro-oxidant qualities, and bioavailability, selenite's use has been questioned by the industry (Nordberg *et al.*, 2022). Selenium serves a significant element as an immunostimulant, an antioxidant, and a compensatory in the immune system, growth increments, and regular bodily functioning of fish (Lukaszewicz *et al.*, 2013). Antioxidant and immunological responses are two often used metrics to evaluate the health of aquaculture animals (Ekasari *et al.*, 2014).

An organic selenium derived from yeast, Sel-Plex, Sel-Plex, provides a remarkable supply of selenium with antioxidant properties. Thus, selenium supplementation holds considerable promise for improving the effectiveness of fish and shrimp production. Organic selenium dietary supplements have been shown to increase the length of harvested tilapia while also improving progeny performance, FCR, weight gain, and fry survival rates (SRs) in tilapia breeders.

This investigation was accomplished to judge the potential benefits of Sel-Plex® nutritional additive on the growth outcomes, physiological response, oxidative status, and immunity-linked gene expression in Nile tilapia (*O. niloticus*) fingerlings exposed to bacterial infection with *Aeromonas hydrophila*.

### Materials and Methods

This investigation was executed for 12 weeks in 2021, at the Fish Diseases Department Laboratory, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

#### Experimental diet preparation

Fish meal, wheat bran, soybean meal, yellow corn, minerals, vitamins, and fish oil were used to provide a base (control) diet that was isonitrogenous and isocaloric, with 12.6 MJ/kg of digestible energy and 30% crude protein, respectively (made in Kafr El-Sheikh, Egypt; at ALEKHWA® feed factory) (Table 1).

Using a feed processor, the dry materials were ground into tiny particles. In the second, third, and fourth diets, rice bran was used as a filler and Sel-Plex® 1000 (Alltech Inc., Nicholasville, KY) was added at a rate of 0.5, 1, and 2 mg/kg to the baseline diet, respectively. Oil, water, dicalcium phosphate, mineral mixture, and vitamins were included in the components after they had been thoroughly combined. The dough was then put through a pelleting extruding machine using a die that was 2–3 mm in diameter. Pellets were allowed to be air-dried before being placed in a 4°C refrigerator for storage. The nutritional profile of test meals was verified using a standardized procedure (AOAC, 2012). The commercial prebiotic product, Sel-Plex®1000 (Alltech Inc., Nicholasville, KY), is an exclusive Alltech organic form of selenium made by yeast. Made to resemble Mother Nature, it is an outstanding nutritional source of selenium. Sel-Plex® selenium is

**Table 1.** Chemical analysis and composition of the experimental diets (based on dry matter).

Components	Diets			
	T1	T2	T3	T4
Fish meal (72% CP)	110	110	110	110
Soybean meal (45% CP)	360	360	360	360
Wheat bran	200	200	200	200
Yellow corn	60	60	60	60
Rice bran	200	199.5	199	198
Sel-Plex® 1000	0	0.5	1	2
Fish oil	15	15	15	15
Soybean oil	15	15	15	15
Dicalcium phosphate	10	10	10	10
Vitamins mixture <sup>1</sup>	10	10	10	10
Minerals mixture <sup>2</sup>	10	10	10	10
Carboxymethyl cellulose	10	10	10	10
Total	1000	1000	1000	1000
Chemical analysis				
Dry matter	91.5	91.3	91.8	91.2
Crude protein	31.3	31.3	31.3	31.3
Ether extract	8.1	8.1	8.1	8.1
Total ash	7.33	7.37	7.3	7.31
Crude fiber	6.6	6.68	6.65	6.62
Nitrogen-free extract	46.7	46.4	46.7	46.5
Gross energy (kcal/g) <sup>3</sup>	4.71	4.72	4.74	4.7

<sup>1</sup> Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

<sup>2</sup> Mineral premix (g/kg of premix): CaHPO<sub>4</sub>·2H<sub>2</sub>O, 727.2; MgCO<sub>4</sub>·7H<sub>2</sub>O, 127.5; KCl 50.0; NaCl, 60.0; FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·3H<sub>2</sub>O, 25.0; ZnCO<sub>3</sub>, 5.5; MnCl<sub>2</sub>·4H<sub>2</sub>O, 2.5; Cu(OAc)<sub>2</sub>·H<sub>2</sub>O, 0.785; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.477; CaIO<sub>3</sub>·6H<sub>2</sub>O, 0.295; CrCl<sub>3</sub>·6H<sub>2</sub>O, 0.128; AlCl<sub>3</sub>·6H<sub>2</sub>O, 0.54; Na<sub>2</sub>SeO<sub>3</sub>, 0.03.

<sup>3</sup> Gross energy (GE) was calculated from NRC (2011) as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively.

safer and more efficient in meeting the greater demand for livestock raised for quick development, reproductive performance, and health.

**Experimental design**

Through a local personal fish hatchery in the Kafr El-Sheikh Governorate, Egypt, 240 healthy Nile tilapia (*O. niloticus*) fingerlings (beginning body weight,  $19.00 \pm 3.00$  g) were provided and brought to the Fish Diseases Laboratory, Faculty of Veterinary Medicine, Kafrelsheikh University. All gathered fish were accommodated in a fiberglass aquarium. Two weeks before the experiment, a baseline diet was introduced to fish (containing 30% dietary protein content).

Following the accommodation time, fish fingerlings were split into 4 groups at random of 60 fingerlings each, and each group was given 3 replicas, each containing 20 fingerlings. Fingerlings were placed in glass tanks that measured  $60 \times 30 \times 40$  cm, held 20 fish per aquarium, and had an efficient aeration system. The aquariums also contained 70 l of water. Group 1 (the control group) was given commercial basal diets, whereas Groups 2–4 were fed meals enhanced with 0.5, 1, and 2 mg/kg of Sel-Plex®.

Fish were given the prescribed meals for 8 weeks at a rate of 3% of the total biomass in each aquarium. Every 2 weeks over the investigation stage, the fish were weighed, and variations in live body weight were taken into consideration while adjusting the feed amounts. Siphoning was used to remove fish waste and feeding waste, and every day, nearly half of each tank’s water level was replenished with dechlorinated fresh water.

Twice a week, water parameters were checked utilizing a water analysis instrument (Lamotte device, USA) according to APHA (1989). During the trial period, the following conditions were observed: temperature between (24°C–27°C), dissolved oxygen  $6.5 \pm 0.5$  mg l<sup>-1</sup>, pH  $7.1 \pm 0.8$ , EC  $219 \pm 2$  μ mho/cm, ammonia adjusted to the typical acceptable limits (<0.1 mg total ammonia), and day and night photoperiod 12:12 hours (Table 2).

**Determination of fish growth parameters**

Fish were fully weighed using an electronic balance after 8 weeks (20 fish/each replicate).

The following parameters; average daily gain (ADG), specific growth rate (SGR), FCR, total weight gain, protein efficiency ratio, and SR%; were determined according to Magouz *et al.* (2019).

**Hematological and biochemical parameters**

Before the final sampling, treated fish were starved for 24 hours. A dose of 100 mg/l of tricaine methane-sulfonate was used to anesthetize fish for hematological assessment to minimize stress during sampling (Dawood *et al.*, 2020b). Nine fish from each group were chosen at random and weighed (three fish/replicate). Feldman *et al.* (2000) report that 5 ml gauge syringes can be utilized to extract blood from the caudal blood vessels. The caudal vein was used to collect blood samples, which were then divided in half. The other blood half was used right away for hematological analysis and differential leukocyte count after being maintained in EDTA-heparinized tubes. According to Urbinate and Carneiro (2006), blood samples were taken from 3 to 4 fish because of the tiny size of the fish. A hemocytometer was used to measure the number of white blood cells (WBCs), and red blood cells (RBCs) in the blood (Houston, 1990). The method of Zinkl (1986) was conducted to determine hemoglobin concentration (Hb g/dl). Decie and Lewis (2006) described the micro-hematocrit method for estimating packed cell volume (PCV%). According to Winthrope’s (1934) description, the mean corpuscular hemoglobin concentration (MCHC) was calculated. To directly quantify the mean corpuscular volume (MCV), an automated Coulter LH 750 hematology analyzer (Beckman Coulter, Fullerton, CA) was utilized (Dacie and Lewis, 1995).

To extract serum, the persisted blood was maintained in non-heparinized tubes. Serum was isolated and maintained at -20°C for subsequent analysis after the blood samples underwent centrifugation at 1,008 g for 15 minutes at 4°C (SCIOLOGEX, Model: DM0412, USA) after 2 hours. According to Thrall *et al.* (2012),

**Table 2.** Water quality metrics were used in this investigation.

Parameters	T1	T2	T3	T4
	Control	Sel-Plex® 0.5% (0.5 mg/kg Feed)	Sel-Plex® 1% (1 mg/kg Feed)	Sel-Plex® 2% (2 mg/kg Feed)
Salinity (ppt)	10	10	10	10
Temperature (°C)	24.3 ± 0.32 <sup>b</sup>	25.9 ± 0.09 <sup>a</sup>	26.3 ± 0.03 <sup>a</sup>	26.9 ± 0.02 <sup>a</sup>
pH	7.8 ± 0.01 <sup>a</sup>	7.9 ± 0.62 <sup>a</sup>	8.02 ± 0.31 <sup>a</sup>	8.02 ± 0.48 <sup>a</sup>
Dissolved Oxygen (mg/l)	6.5 ± 0.81 <sup>a</sup>	6.4 ± 0.33 <sup>a</sup>	6.8 ± 0.32 <sup>a</sup>	7.1 ± 0.23 <sup>a</sup>
NH3 (mg/l)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01
NH4	0.25 ± 0.32 <sup>b</sup>	0.38 ± 0.48 <sup>a</sup>	0.39 ± 0.81 <sup>a</sup>	0.41 ± 0.73 <sup>a</sup>
NO <sub>2</sub>	0.042 ± 0.43 <sup>a</sup>	0.037 ± 0.22 <sup>a</sup>	0.040 ± 0.52 <sup>a</sup>	0.046 ± 0.62 <sup>a</sup>

differential WBC counts were performed. It was air dried, when a thin blood film was obtained, methanol fixed for 3–5 minutes, stained with Geimsa stain for 10–12 minutes, rinsed with purified water and allowed to dry. According to Stoskopf (1993), the WBCs were enumerated among the blood smear's 100 components. Serum albumins and total proteins were measured following Dumas and Biggs (1972). Globulin is computed as the difference between readings of total protein and albumin.

Diagnostic commercial kits (Biodiagnostic Co., Egypt) were utilized in accordance with the manufacturer's directions to measure alanine aminotransferase (ALT), and serum aspartate aminotransferase (AST) (Reitman and Frankel, 1957), urea (Fawcett and Soctt, 1960), uric acid, and creatinine (Bartels and Bohmer, 1972).

#### Determination of immune parameters

- a-. Phagocytic activity (PA) and phagocytic index (PI): PA was ascertained in accordance with Kawahara *et al.* (1991). The PI was also calculated.
- b-. Lysozyme activity in serum:
- c-. Utilizing the protocol stated by Demers and Bayne (1997), lysozyme activity was examined in light of the Gram-positive lysozyme delicate lyophilized bacteria, *Micrococcus lysodeikticus* (Sigma, St. Louis, MO).
- d-. Superoxide dismutase (SOD): Colorimetric analysis was utilized in accordance with Nishikimi *et al.* (1972).
- e-. Catalase (CAT) activity was completed by verifying hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) using spectrophotometry, as previously instructed (Aebi, 1984).
- f-. Glutathione peroxidase (GPx) was established by applying the protocol used by Prieto *et al.* (1999).
- g-. Lipid peroxide (Malondialdehyde, MDA) was measured using colorimetric methods after Ohkawa *et al.* (1979).

#### The challenge with *A. hydrophila*

The goal of the experiment was to ascertain the impact of adding Sel-Plex<sup>®</sup> to the diet on *O. niloticus* fingerlings' resistance to *A. hydrophila* bacterial infection. According to Li *et al.* (2011), an experimental infection

was performed via an intra-peritoneal injection of 0.2 ml of fresh *A. hydrophila* culture suspension (3 × 10<sup>7</sup> cfu). Along 15 days after infection, everyday mortalities were noted.

Following Cruickshank (1975), the concentration of the *A. hydrophila* strain per 1 ml and the dose for the experimental experiments were estimated utilizing the drop plate technique. The *A. hydrophila* strain was cultured for 24 hours on Tryptic Soy Agar (TSA) plates; the colonies were taken up and suspended in sterile saline using a 10-fold serial dilution. The next 24 hours were then incubated at 28°C to count the colonies on the TSA plate. The dilutions (10<sup>2</sup> to 10<sup>7</sup> CFU) only were just utilized.

Every group received an intraperitoneal injection of 0.5 ml/fish of every bacterial dilution. For 2 weeks following injection, all of the fish were maintained for observation. According to Ibrahim *et al.* (2011), the mortalities were noted two times daily. Dead fish were transported for additional PM inspection. According to Reed and Muench (1938), LD<sub>50</sub>, which refers to the lethal dose that results in the mortality of 50% of the administered fish, was computed.

#### Gene expression analysis

In accordance with the manufacturer's guidelines, the extraction of total RNA was carried out using the TRIzol reagent (Life Technologies, Gaithersburg, MD). The cDNA synthesis was performed immediately using the MultiScribe RT enzyme kit (Applied Biosystems, Foster City, CA). The cDNA obtained was subjected to real-time polymerase chain reaction (PCR) analysis, which was conducted in triplicate. Real-time PCR experiments were conducted using Power SYBR Green PCR Master Mix on a 7500 real-time PCR system manufactured by Applied Biosystems in Foster City, CA. Different genes' relative fold changes in mRNA expression were assessed in relation to the control. The normalization of the fold change in mRNA expression of the measured genes was performed by utilizing the expression of β-actin, which is a widely used housekeeping gene. Table 3 presents the gene accession codes and primer sequences.

#### Histopathological examinations

For histopathological examinations, tissue samples from the anterior part of the intestine, hepatopancreas,

**Table 3.** Gene primer sequences used in real-time PCR analysis.

Target genes	Forward primer	Reverse primer	Accession No.
β-Actin	CAGCAAGCAGGAGTACGATGAG	TGTGTGGTGTGTGGTTGTTTTG	EU887951.1
IL-1B	CAAGGATGACGACAAGCCAACC	AGCGGACAGACATGAGAGTGC	XM_003460625.2
TNF-α	GGAAGCAGCTCCACTCTGATGA	CACAGCGTGTCTCCTTCGTTCA	JF957373.1
SOD	CCCTACGTCAAGTGCAGAGAT	GTCACGTCTCCCTTTGCAAG	JF801727.1
GPX	CGCCGAAGGTCTCGTTATTT	TCCCTGGACGGACATACTT	NM_001279711.1
IGF-1	GTTTGTCTGTGGAGAGCGAGG	GAAGCAGCACTCGTCCACG	XM_00344805
GHR	CAGACTTCTACGCTCAGGTC	CTGGATTCTGAGTTGCTGTC	AY973232.1

kidney, and spleen were taken immediately after anesthesia using 40% ethyl alcohol from randomly selected five fish from each treatment (0, 0.5, 1, and 2 mg of Sel-Plex®1000) pre and post-challenge with *A. hydrophila*. The specimens were preserved in 10% formaldehyde solution for 24 hours, and then dehydrated by using ascending concentrations of ethanol (70% to absolute alcohol). The dehydrated samples were cleared in xylene and embedded in paraffin wax. For histopathological and morphometric analysis, thin sections measuring 4–5 µm in thickness were stained utilizing hematoxylin and eosin according to Moustafa et al. (2020).

#### Morphometry of intestinal villi

The dimensions of intestinal villi, including length and width, as well as the depth of crypts and the surface area of villi, were quantified using image analysis software provided by the National Institutes of Health, Bethesda, MD. A selection of 10 random villi and villus-associated crypts were chosen from 5 cross-sections of the intestine. The resulting data was subjected to statistical analysis using a one-way analysis of variance (ANOVA) in SPSS version 22 (SPSS Inc., Chicago, IL). The chosen level of significance was deemed acceptable at a threshold of  $p < 0.05$ . The data are reported in the form of means  $\pm$  standard error (SE).

#### Statistical analysis

The study utilized a two-way ANOVA to examine the impact of dietary Sel-Plex and challenge conditions, as well as their potential interactions. After establishing an interaction between the two factors, a one-way ANOVA was conducted to examine the combined effect of all levels of the two factors on the four groups.

The data obtained from each group were subjected to comparison with the control group. Differences within the same group and among the different sample times for each parameter were assessed using the Tukey–Kramer method. Statistical differences were evaluated using one-way ANOVA tests, conducted with SPSS version 22 (SPSS Inc., Chicago, IL). In cases where differences were observed among the experimental groups, the Duncan *post-hoc* test was employed. The chosen level of significance was deemed acceptable at a threshold of  $p < 0.05$ . The data are reported as means  $\pm$  standard error (SE).

#### Ethical approval

All fish handling procedures were executed in correspondence to the animal care and use criteria established by the Ethics Committee for scientific purposes, at Kafrelsheikh University, Egypt (KFS-IACUC/138/2023).

### Results

The present investigation examines the physiological responses of *O. niloticus* fingerlings to Sel-Plex®1000 was evaluated via monitoring of fish growth, and hematological, biochemical, and immunological parameters.

#### Growth performance

In the present investigation, *O. niloticus* growth responses to Sel-Plex®1000 were examined. It was found that all groups that received treatment exhibited increases in ADG, weight gain, SGR, final fish weight, weight gain %, feed intake, and fish biomass compared to the control group (T1) ( $p < 0.05$ ), as shown in Table 4. In addition, the impact of Sel-Plex®1000 on

**Table 4.** The effect of Sel-Plex®1000 on growth performance of *O. niloticus*.

Growth performance	T1	T2	T3	T4
Initial fish weight(g)	18.97 $\pm$ 0.12 <sup>a</sup>	18.87 $\pm$ 0.07 <sup>a</sup>	19.13 $\pm$ 0.09 <sup>a</sup>	19.03 $\pm$ 0.09 <sup>a</sup>
Final fish weight (g)	31.43 $\pm$ 0.16 <sup>b</sup>	36.64 $\pm$ 0.55 <sup>a</sup>	37.52 $\pm$ 0.45 <sup>a</sup>	36.10 $\pm$ 0.75 <sup>a</sup>
Weight gain	12.47 $\pm$ 0.27 <sup>b</sup>	17.77 $\pm$ 0.58 <sup>a</sup>	18.38 $\pm$ 0.51 <sup>a</sup>	17.07 $\pm$ 0.77 <sup>a</sup>
Weight gain %	65.75 $\pm$ 1.84 <sup>b</sup>	94.20 $\pm$ 3.27 <sup>a</sup>	96.10 $\pm$ 2.94 <sup>a</sup>	89.68 $\pm$ 4.14 <sup>a</sup>
SGR %/fish/day	1.12 $\pm$ 0.02 <sup>b</sup>	1.48 $\pm$ 0.04 <sup>a</sup>	1.49 $\pm$ 0.03 <sup>a</sup>	1.42 $\pm$ 0.05 <sup>a</sup>
Feed intake	25.63 $\pm$ 0.05 <sup>c</sup>	26.89 $\pm$ 0.08 <sup>b</sup>	27.48 $\pm$ 0.12 <sup>a</sup>	27.32 $\pm$ 0.11 <sup>a</sup>
FCR	2.06 $\pm$ 0.05 <sup>a</sup>	1.52 $\pm$ 0.04 <sup>b</sup>	1.50 $\pm$ 0.03 <sup>b</sup>	1.61 $\pm$ 0.06 <sup>b</sup>
ADG	28.00 $\pm$ 0.58 <sup>b</sup>	39.33 $\pm$ 1.33 <sup>a</sup>	41.00 $\pm$ 1.15 <sup>a</sup>	37.67 $\pm$ 1.67 <sup>a</sup>
Fish biomass	628.67 $\pm$ 3.28 <sup>b</sup>	732.73 $\pm$ 10.95 <sup>a</sup>	750.33 $\pm$ 9.02 <sup>a</sup>	722.00 $\pm$ 15.04 <sup>a</sup>
Initial number	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00
Final number	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00	20.00 $\pm$ 0.00
Fish survival (%)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00

The data represent the mean  $\pm$  SE. The values with different superscripts in the same row indicate significant differences ( $p < 0.05$ ). T1= (control group), T2= (0.5% Sel-Plex®), T3= (1% Sel-Plex®), T4= (2 % Sel-Plex®), S.G.R = Specific growth rate, ADG = Average daily gain, FCR=Feed conversion rate.

FCR was summarized. It was noticed that, contrasted with the control group (T1), the supplemented groups had the best food conversion rate values ( $p < 0.05$ ) (Table 4). The rate of SR% was shown to be 100% across all groups.

**Hematological parameters**

In all treatment groups, there was a substantial increase in Hb, RBCs, and MCH ( $p < 0.05$ ). In comparison to T1, PCV greatly rose in T3 and T4, while it dramatically dropped in T2. MCV significantly increased ( $p < 0.05$ ) in the T4 group. In comparison with the control group, decreased ( $p < 0.05$ ) in the T2 group contrasted with T1, and there was no noticeable difference among the T3 and T1 groups. MCHC increased noticeably ( $p < 0.05$ ) in all treated groups with the greatest value in T2. WBCs considerably ( $p < 0.05$ ) increased in the T2 and T3 groups contrasted with the T1 group, but not noticeably ( $p < 0.05$ ) in the T4 group (Table 5). Regarding differential leukocytic count, the observed data revealed that heterophils and eosinophil (%)

increased considerably ( $p < 0.05$ ) in the augmented groups when comparing the control group, while lymphocytes (%) exhibited no changes in the augmented groups contrasted with the control group. Monocyte (%) was increased significantly in the T2 and T4 groups, while the T3 group showed no observed differences between contrasted with the control group. Basophil (%) was reduced considerably ( $p < 0.05$ ) in all supplemented groups in comparison to the control group (Table 6).

**Biochemical analysis**

According to the data shown in Table 7, ALT level expressed a noticeable decline ( $p < 0.05$ ), in all supplemented groups, while no changes were observed in AST level across all groups. Albumin, total protein, and globulin levels exhibited a noticeable increase in all supplemented groups contrasted with the control group ( $p < 0.05$ ). The outcomes for uric acid revealed a considerable drop ( $p < 0.05$ ), in all supplemented groups contrasted with the control group, while urea and creatinine in all groups remained unchanged.

**Table 5.** Sel-Plex®1000 Effect on hematological parameters of *O. niloticus*.

Parameters	T1	T2	T3	T4
RBCs (x10/mm <sup>3</sup> )	2.28 ± 0.01 <sup>b</sup>	2.33 ± 0.02 <sup>a</sup>	2.44 ± 0.04 <sup>a</sup>	2.46 ± 0.01 <sup>a</sup>
Hb (g/100ml)	6.37 ± 0.40 <sup>c</sup>	6.93 ± 0.10 <sup>b</sup>	7.11 ± 0.10 <sup>a</sup>	7.05 ± 0.16 <sup>b</sup>
PCV (%)	21.33 ± 0.88 <sup>b</sup>	20.67 ± 0.33 <sup>c</sup>	22.67 ± 0.33 <sup>a</sup>	23.00 ± 0.58 <sup>a</sup>
MCV	92.65 ± 0.05 <sup>b</sup>	88.19 ± 0.47 <sup>c</sup>	92.21 ± 0.35 <sup>b</sup>	93.62 ± 2.04 <sup>a</sup>
MCH	27.98 ± 0.48 <sup>b</sup>	29.72 ± 0.71 <sup>a</sup>	29.44 ± 0.10 <sup>a</sup>	29.08 ± 0.53 <sup>a</sup>
MCHC	30.36 ± 0.46 <sup>b</sup>	33.95 ± 0.96 <sup>a</sup>	31.86 ± 0.03 <sup>a</sup>	31.04 ± 0.15 <sup>a</sup>
WBCs (x10 <sup>3</sup> /mm <sup>3</sup> )	12.59 ± 1.14 <sup>b</sup>	13.91 ± 1.15 <sup>a</sup>	13.38 ± 0.55 <sup>a</sup>	12.39 ± 0.84 <sup>b</sup>

The data represent the mean ± SE. The values with various superscripts in the same row indicate significant differences ( $p < 0.05$ ). T1= (control group), T2= (0.5% Sel-Plex®), T3= (1% Sel-Plex®), T4= (2 % Sel-Plex®), RBCs= Red Blood Cells, HB = Haemoglobin, PCV = Packed Cell Volume, MCV= Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC =Mean corpuscular hemoglobin concentration, WBCs= White Blood Cells.

**Table 6.** Sel-Plex®1000 Effect on the differential leukocytic count of *O. niloticus*.

Parameters	T1	T2	T3	T4
Heterophil (%)	13.33 ± 0.33 <sup>a</sup>	9.00 ± 0.58 <sup>b</sup>	9.67 ± 0.33 <sup>b</sup>	9.67 ± 0.33 <sup>b</sup>
Lymphocyte (%)	76.67 ± 0.33 <sup>a</sup>	81.67 ± 0.88 <sup>a</sup>	83.00 ± 0.00 <sup>a</sup>	81.00 ± 1.73 <sup>a</sup>
Monocyte (%)	6.33 ± 0.33 <sup>b</sup>	8.67 ± 0.33 <sup>a</sup>	6.67 ± 0.33 <sup>b</sup>	8.00 ± 0.58 <sup>a</sup>
Eosinophil (%)	0.67 ± 0.33 <sup>a</sup>	0.77 ± 0.33 <sup>b</sup>	1.00 ± 0.00 <sup>b</sup>	0.77 ± 0.33 <sup>b</sup>
Basophil (%)	1.67 ± 0.67 <sup>a</sup>	0.67 ± 0.33 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	1.00 ± 0.58 <sup>b</sup>
Heterophil	1.07 ± 0.00 <sup>a</sup>	1.31 ± 0.18 <sup>b</sup>	1.27 ± 0.01 <sup>b</sup>	1.20 ± 0.03 <sup>b</sup>
Lymphocyte	10.24 ± 0.01 <sup>a</sup>	11.58 ± 0.77 <sup>a</sup>	11.12 ± 0.46 <sup>a</sup>	10.34 ± 0.84 <sup>a</sup>
Monocyte	0.75 ± 0.03 <sup>b</sup>	1.20 ± 0.05 <sup>a</sup>	0.88 ± 0.07 <sup>b</sup>	1.01 ± 0.01 <sup>a</sup>
Eosinophil	0.17 ± 0.01 <sup>b</sup>	0.08 ± 0.05 <sup>ab</sup>	0.13 ± 0.01 <sup>b</sup>	0.06 ± 0.03 <sup>ab</sup>
Basophil	0.19 ± 0.01 <sup>a</sup>	0.08 ± .05 <sup>c</sup>	0.00 ± 0.00 <sup>d</sup>	0.12 ± 0.07 <sup>ab</sup>

The data represent the mean ± SE. The values with various superscripts in the same row indicate significant differences ( $p < 0.05$ ). T1= (control group), T2= (0.5% Sel-Plex®), T3= (1% Sel-Plex®), T4= (2 % Sel-Plex®).

**Table 7.** Sel-Plex®1000 Effect on biochemical analysis of *O. niloticus*.

Parameters	T1	T2	T3	T4
ALT (U/l)	7.05 ± 0.00 <sup>a</sup>	6.70 ± 0.03 <sup>b</sup>	6.79 ± 0.00 <sup>b</sup>	6.96 ± 0.03 <sup>b</sup>
AST (U/l)	70.37 ± 0.00 <sup>a</sup>	67.20 ± 1.70 <sup>a</sup>	64.72 ± 2.79 <sup>a</sup>	66.12 ± 0.38 <sup>a</sup>
Total Protein (g/dl)	2.86 ± 0.01 <sup>b</sup>	3.01 ± 0.04 <sup>a</sup>	3.03 ± 0.02 <sup>a</sup>	3.03 ± 0.01 <sup>a</sup>
Albumin (g/dl)	1.14 ± 0.01 <sup>b</sup>	1.14 ± 0.00 <sup>a</sup>	1.16 ± 0.00 <sup>a</sup>	1.16 ± 0.03 <sup>a</sup>
Globulin (g/dl)	1.72 ± 0.00 <sup>b</sup>	1.87 ± 0.03 <sup>a</sup>	1.87 ± 0.02 <sup>a</sup>	1.87 ± 0.04 <sup>a</sup>
Urea (mg/dl)	4.06 ± 0.01 <sup>a</sup>	4.03 ± 0.10 <sup>a</sup>	4.00 ± 0.00 <sup>a</sup>	3.92 ± 0.08 <sup>ab</sup>
Creatinine (mg/dl)	0.31 ± 0.01 <sup>a</sup>	0.26 ± 0.02 <sup>a</sup>	0.27 ± 0.01 <sup>a</sup>	0.31 ± 0.00 <sup>a</sup>
Uric acid (mg/dl)	1.83 ± 0.00 <sup>a</sup>	1.82 ± 0.03 <sup>ab</sup>	1.68 ± 0.05 <sup>b</sup>	1.72 ± 0.01 <sup>b</sup>

The data represent the mean ± SE. The values with various superscripts in the same row indicate significant differences ( $p < 0.05$ ). T1= (control group), T2= (0.5% Sel-Plex®), T3= (1% Sel-Plex®), T4= (2 % Sel-Plex®), ALT = Alanine aminotransferase, AST=Aspartate aminotransferase.

**Table 8.** Sel-Plex®1000 Effect on immunity and oxidative status of *O. niloticus*.

Parameters	T1	T2	T3	T4
PA	8.72 ± 0.01 <sup>ab</sup>	9.22 ± 0.02 <sup>ab</sup>	9.36 ± 0.08 <sup>a</sup>	9.21 ± 0.01 <sup>ab</sup>
PI	1.22 ± 0.01 <sup>b</sup>	1.53 ± 0.14 <sup>a</sup>	1.54 ± 0.11 <sup>a</sup>	1.54 ± 0.06 <sup>a</sup>
lysozyme (u/ml)	8.67 ± 0.01 <sup>b</sup>	9.01 ± 0.07 <sup>ab</sup>	9.23 ± 0.01 <sup>a</sup>	9.10 ± 0.05 <sup>a</sup>
SOD (IU/l)	12.30 ± 0.03 <sup>c</sup>	13.25 ± 0.05 <sup>ab</sup>	13.94 ± 0.25 <sup>a</sup>	13.72 ± 0.08 <sup>ab</sup>
CAT (IU/l)	20.21 ± 0.01 <sup>b</sup>	20.48 ± 0.04 <sup>a</sup>	20.74 ± 0.07 <sup>a</sup>	20.74 ± 0.25 <sup>a</sup>
GPX (IU/l)	24.29 ± 0.05 <sup>c</sup>	25.87 ± 0.07 <sup>b</sup>	26.44 ± 0.06 <sup>a</sup>	26.03 ± 0.23 <sup>a</sup>
MDA (IU/l)	31.38 ± 0.01 <sup>a</sup>	30.23 ± 0.12 <sup>b</sup>	29.15 ± 0.34 <sup>c</sup>	29.17 ± 0.07 <sup>c</sup>

The data represent the mean ± SE. The values with various superscripts in the same row indicate significant differences ( $p < 0.05$ ). T1= (control group), T2= (0.5% Sel-Plex®), T3= (1% Sel-Plex®), T4= (2% Sel-Plex®), SOD=Superoxide dismutase, CAT=Catalase, GPX = Glutathione peroxidase, MDA= Malondialdehyde.

#### Immunological and antioxidant parameters

Concerning the immunological measures, there was no change among the T2, T4 groups, and the control group; however, PA was dramatically increased in the T3 group compared to the control group ( $p < 0.05$ ). The PI was increased in all supplemented groups in comparison to the control group ( $p < 0.05$ ). According to Table 8, all supplemented groups' lysozyme activity significantly increased compared with the control group ( $p < 0.05$ ). The antioxidant activity showed that CAT, SOD, and GPx activities were raised in all augmented groups with the highest value in the T3 group in comparison to the control group ( $p < 0.05$ ). MDA levels were noticeably decreased in the T3 and T4 groups ( $p < 0.05$ ) (Table 8).

#### Antioxidant activity after experimental challenge with *A. hydrophila*

Immunological measures such as PA, PI, and lysozyme activity were noticeably increased in all supplemented groups in comparison to the control group ( $p < 0.05$ ). The antioxidant activity showed that CAT, SOD, and GPx activities were raised in all supplemented groups with the highest value in the T3 group contrasted with the control group ( $p < 0.05$ ). MDA activity was noticeably decreased in all supplemented groups ( $p <$

0.05). The *A. hydrophila* challenged groups revealed a 100% SR mainly among the Sel-Plex® supplemented groups (Table 9).

#### Relative gene expression

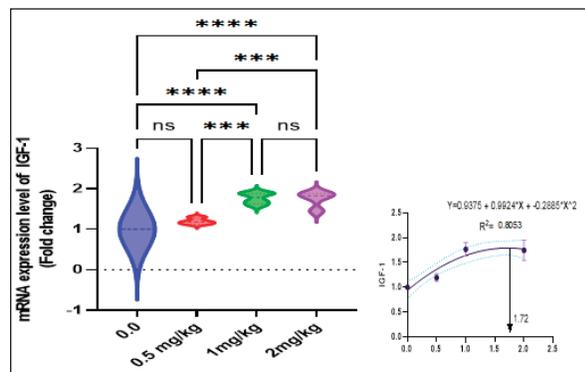
Liver showed upregulation of mRNA expression of insulin-like growth factor 1 (IGF-1), in T3 and T4 groups treated with 1 and 2 mg/kg Sel-Plex®1000 contrasted with other treated groups ( $p < 0.05$ ), while there were no noticeable differences between T3 and T4 groups (Fig. 1), while liver showed upregulation of mRNA expression of growth hormone receptor (GHR) in all supplemented groups contrasted with the control group ( $p < 0.05$ ) and no noticeable variation among T2, T3, and T4 groups (Fig. 2). Pairwise comparisons were conducted with the control group to determine the maximum expression values of the aforementioned genes. It was observed that these maximum expression values were noticed at a dietary concentration of 1.7 mg Sel-Plex /kg. Subsequently, a significant reduction in the expression values of these genes was observed (Fig. 1).

The present study observed a noticeable ( $p < 0.05$ ) rise in the mRNA expression of SOD in the liver samples of all groups fed diets supplemented with Sel-Plex®1000

**Table 9.** Sel-Plex®1000 Effect on immunological and oxidative status of *O. niloticus* after challenge with *A. hydrophila*.

Parameters	T1	T2	T3	T4
PA	8.74 ± 0.01 <sup>b</sup>	9.29 ± 0.03 <sup>a</sup>	9.41 ± 0.01 <sup>a</sup>	9.40 ± 0.02 <sup>a</sup>
PI	1.22 ± 0.00 <sup>b</sup>	1.55 ± 0.11 <sup>a</sup>	1.57 ± 0.13 <sup>a</sup>	1.60 ± 0.06 <sup>a</sup>
lysozyme (u/ml)	7.83 ± 0.02 <sup>b</sup>	9.05 ± 0.12 <sup>a</sup>	9.02 ± 0.09 <sup>a</sup>	9.10 ± 0.12 <sup>a</sup>
SOD (IU/l)	12.74 ± 0.01 <sup>b</sup>	13.60 ± 0.17 <sup>a</sup>	13.65 ± 0.19 <sup>a</sup>	13.87 ± 0.26 <sup>a</sup>
CAT (IU/l)	20.18 ± 0.00 <sup>b</sup>	22.25 ± 0.27 <sup>a</sup>	23.62 ± 0.55 <sup>a</sup>	22.59 ± 0.22 <sup>a</sup>
GPX (IU/l)	24.76 ± 0.21 <sup>c</sup>	27.40 ± 0.13 <sup>a</sup>	28.01 ± 0.22 <sup>a</sup>	26.20 ± 0.19 <sup>b</sup>
MDA (IU/l)	33.52 ± 0.55 <sup>a</sup>	28.88 ± 0.05 <sup>c</sup>	28.65 ± 0.23 <sup>c</sup>	29.49 ± 0.14 <sup>b</sup>
SR	50%	100%	100%	100%

The data represent the mean ± SE. The values with various superscripts in the same row indicate significant differences ( $p < 0.05$ ). T1= (control group), T2= (0.5% Sel-Plex®), T3= (1% Sel-Plex®), T4= (2% Sel-Plex®), SOD=Superoxide dismutase, CAT=Catalase, GPX = Glutathione peroxidase, MDA= Malondialdehyde.



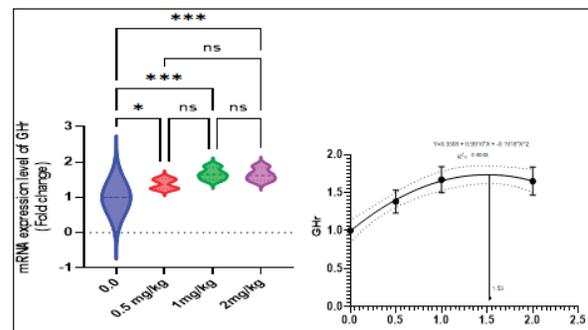
**Fig. 1.** Gene expression of the liver (IGF-1) of Nile tilapia in different groups fed diets with different doses of Sel-Plex®1000 for 8 weeks. \*indicate significant differences between groups ( $p < 0.05$ ), ns = indicate no significant differences between groups.

with the highest level in T3 and T4 groups, while there was no noticeable variation between T3 and T4 groups (Fig. 3), while GPx mRNA expression in the liver samples exhibited a substantial difference noticeably ( $p < 0.05$ ) increased in T3 and T4 groups treated with 1 and 2 mg/kg Sel-Plex®1000 in comparison with other treated groups, while no substantial variation between T3 and T4 groups was observed (Fig. 4).

It is noticed that the mRNA expression of interleukin 1 $\beta$  (IL-1 $\beta$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ) in the liver samples were substantially ( $p < 0.05$ ) increased in all groups augmented with Sel-Plex®1000, while no substantial variation between T3 and T4 groups before and post challenge with *A. hydrophila* (Figs. 5 and 6).

#### Histopathological findings

The morphometric analysis of the intestine of pre-challenged groups showed a marked increase in all measured parameters; villi length, width, crypt depth, villi length/crypt depth, and villi surface area in a dose-dependent pattern especially 1 and 2 mg Sel-Plex®1000 groups, than the other treated groups as illustrated in

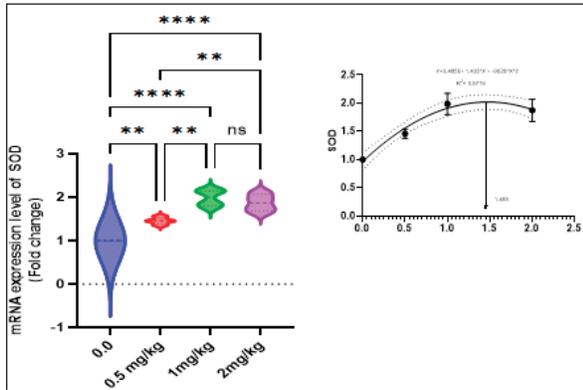


**Fig. 2.** Gene expression of the liver (GHR) of Nile tilapia in different groups fed diets with different doses of Sel-Plex®1000 for 8 weeks. \*indicate significant differences between groups ( $P < 0.05$ ), ns = indicate no significant differences between groups.

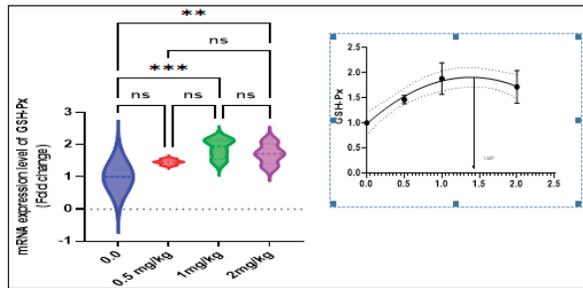
Table 10 besides, increased branching of intestinal villi in comparison to the other groups (Fig. 7).

The histopathological examination of the intestine of fish challenged with *A. hydrophila* showed severe necrotic changes and sloughing of villi epithelium which include most of the intestinal villi length in the 0.0 mg Sel-Plex®1000 group. The adverse effect diminished with an increase in the concentration of Sel-Plex®1000 (0.5 and 1 mg groups) while the 2 mg Sel-Plex®1000 group showed intact villi with a mild increase in villi length and branching in comparison to the previously mentioned groups. In contrast, the non-challenged groups showed intact villi with an increase in both length and branching in a way dependent on dosage (Fig. 7).

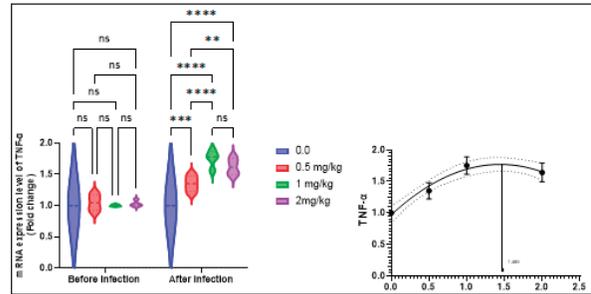
The hepatopancreas of pre-challenged groups shows normal architecture of the liver with polyhedral-shaped hepatocytes separated by hepatic sinusoids and intact pancreatic acini while the hepatopancreas of 0 mg Sel-Plex®1000 challenged group show zones of necrosis in both hepatic and pancreatic tissue beside severe vacuolar degeneration and nuclear pyknosis. The other



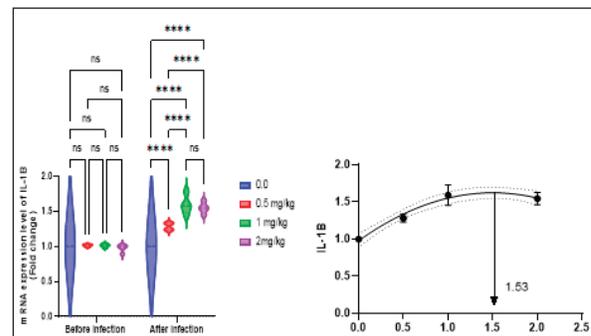
**Fig. 3.** Gene expression of the liver (SOD) of Nile tilapia in different groups fed diets with different doses of Sel-Plex®1000 for 8 weeks. \*indicate significant differences between groups ( $P < 0.05$ ), ns = indicate no significant differences between groups.



**Fig. 4.** Gene expression of the liver (GSH-Px) of Nile tilapia in different groups fed diets with different doses of Sel-Plex®1000 for 8 weeks. \*indicate significant differences between groups ( $P < 0.05$ ), ns = indicate no significant differences between groups.



**Fig. 5.** Gene expression of liver (TNF- $\alpha$ ) of Nile tilapia pre and post-challenge with *A. hydrophila* in different groups fed diets with different doses of Sel-Plex®1000 for 8 weeks. \*indicate significant differences between groups ( $P < 0.05$ ), ns = indicate no significant differences between groups.

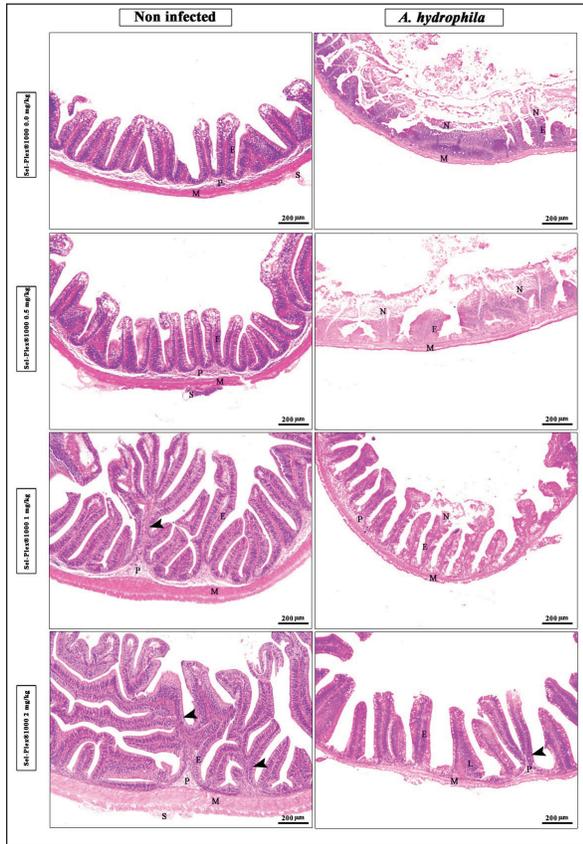


**Fig. 6.** Gene expression of the liver (IL-1 $\beta$ ) of Nile tilapia pre and post challenge with *A. hydrophila* in different groups fed diets with different doses of Sel-Plex®1000 for 8 weeks. \*indicate significant differences between groups ( $P < 0.05$ ), ns = indicate no significant differences between groups.

**Table 10.** Morphometric analysis of the intestine of *O. niloticus* fed on diets containing different levels Sel-Plex®.

Groups	Sel-Plex®1000 0 mg	Sel-Plex®1000 0.5 mg	Sel-Plex®1000 1 mg	Sel-Plex®1000 2 mg	p-value
Parameters					
Villi length ( $\mu\text{m}$ )	194.1 $\pm$ 5.46 <sup>b</sup>	261.5 $\pm$ 6.71 <sup>b</sup>	470.2 $\pm$ 36.72 <sup>a</sup>	502.7 $\pm$ 39.49 <sup>a</sup>	< 0.0001
Crypt depth ( $\mu\text{m}$ )	46.64 $\pm$ 2.03 <sup>ab</sup>	45.22 $\pm$ 3.46 <sup>ab</sup>	54.83 $\pm$ 6.37 <sup>a</sup>	31.77 $\pm$ 2.57 <sup>b</sup>	0.0025
villi width ( $\mu\text{m}$ )	92.17 $\pm$ 4.58	87.35 $\pm$ 5.08	91.90 $\pm$ 3.54	89.48 $\pm$ 5.16	0.2394
Villi length/crypt depth	4.24 $\pm$ 0.22 <sup>c</sup>	6.02 $\pm$ 0.38 <sup>c</sup>	9.30 $\pm$ 1.05 <sup>b</sup>	15.72 $\pm$ 1.18 <sup>a</sup>	< 0.0001
Villi surface area ( $\mu\text{m}^2$ )	18757 $\pm$ 982.2 <sup>c</sup>	19701 $\pm$ 1307 <sup>bc</sup>	29389 $\pm$ 2021 <sup>ab</sup>	34001 $\pm$ 4335 <sup>a</sup>	0.0012

The presented data represent the mean  $\pm$  SE. The values with various superscripts in the same row indicate significant differences ( $p < 0.05$ ).

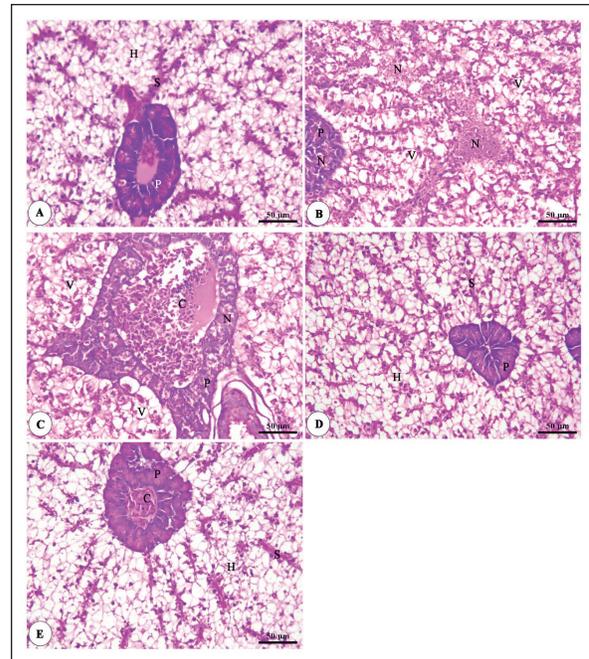


**Fig. 7.** Photomicrograph of H&E stained panel of the anterior part of the intestine of *O. niloticus* fed on ration contained 0, 0.5, 1, and 2 mg of Sel-Plex®1000 pre and post-challenge with *A. hydrophila* showing intestinal villi lined with simple columnar cells of lamina epithelialis (E), lamina propria (P), muscular layer (M), tunica serosa (S), branched alveoli (arrow head), and areas of necrosis and sloughing of intestinal villi (N).

treated groups; 0.5, 1, and 2 mg Sel-Plex®1000 show significant amelioration which ranged from vacuolar degeneration and congestion of blood vessels in the 0.5 mg Sel-Plex®1000 treated group to apparently intact hepatic and pancreatic tissue with mild fatty changes in 2 mg Sel-Plex®1000 treated group (Fig. 8).

Histopathological examination of the posterior part of the fish kidney of pre-challenged groups shows normal renal architecture with intact glomeruli, renal tubules, and interstitial tissue. The kidney of the 0.0% Sel-Plex®1000 *A. hydrophila*-challenged group shows necrosis of some renal tubule, sloughing of the tubular epithelium into the lumen, interstitial edema, and congestion of renal blood vessels. The lesions were markedly diminished with increased Sel-Plex®1000 concentration in a way dependent on dosage from 0.5 to 2 mg (Fig. 9).

The histopathological examinations of the spleen of pre-challenged groups show the normal structure of the



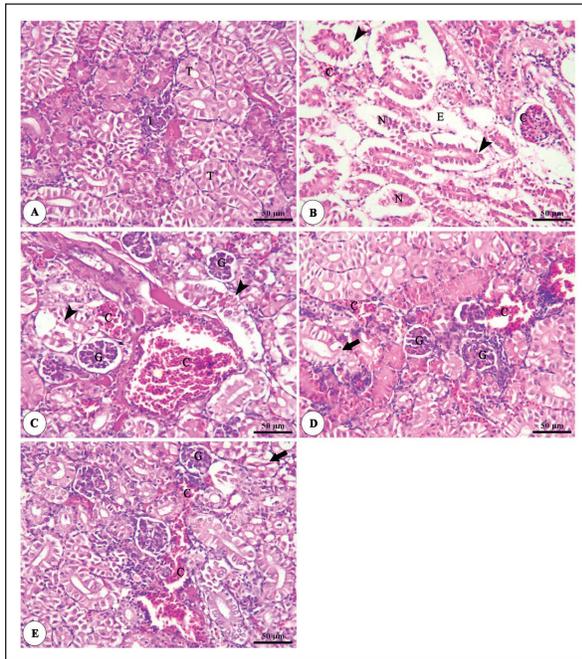
**Fig. 8.** Photomicrograph of H&E stained panel of hepatopancreas of *O. niloticus* pre-challenge (A) and post-challenge with *A. hydrophila* fed on ration contained 0 (B), 0.5 (C), 1 (D) and 2 mg (E) of Sel-Plex®1000 showing hepatocytes (H), blood sinusoids (S), pancreatic acini (P), focal areas of necrosis in hepatic and pancreatic tissue (N), vacuolar degeneration (V), and congestion of blood vessels (C).

spleen with intermingled red and white pulp in addition to melanomacrophage centers. The spleen of the 0 mg Sel-Plex®1000 challenged group shows severe degenerative changes and necrosis, depletion of white pulp, and interstitial edema. The 0.5, 1, and 2 mg Sel-Plex®1000 treated group show a remarkable improvement of the spleen with increased melanomacrophage centers in a dose-dependent manner (Fig. 10).

## Discussion

Aquaculture systems can use probiotics as either feed or water additions (Taoka *et al.*, 2006). The kind and duration of probiotic and prebiotic therapy may have an impact on fish health (Welker and Lim, 2011). The efficacy of the probiotic bacterial settlement in the fish digestive system has been demonstrated to be improved by supplementing meals with pre- and probiotics (Mei *et al.*, 2011).

The aqua feeds industry is primarily concerned with gaining benefits from the improved growth and resistance response of the great majority of variety of cultivated fish, in addition to preventive healthcare services through a variety of healthy protocols to ensure its manageability in the aquatic farming system (Kiron, 2012). The nutritional health of farmed fish species is the primary factor that affects their immune status and,



**Fig. 9.** Photomicrograph of H&E stained panel of kidney of *O. niloticus* pre-challenge (A) and post-challenge with *A. hydrophila* fed on ration contained 0 (B), 0.5 (C), 1 (D) and 2 mg (E) of Sel-Plex®1000 showing renal glomeruli (G), renal tubules (T), interstitial tissue (I), congestion of glomerular and interstitial blood vessels (C), degeneration and separation of renal tubular epithelium from its basement membrane (arrow heads), necrosis of some renal tubules (N), mild vacuolar degeneration and nuclear pyknosis (arrows) and edema in the interstitial tissue (E).

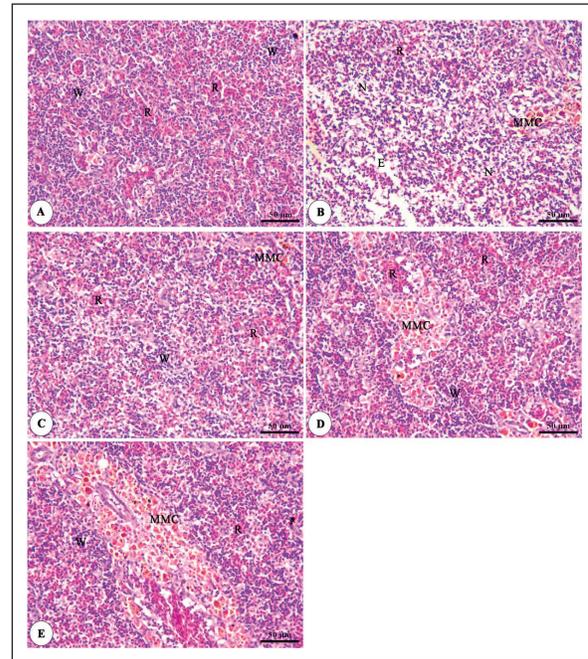
ultimately, helps to achieve a greater level of assurance (Dawood *et al.*, 2018b).

Functional nutritional supplements are a crucial management tool for boosting farmed fish's resistance to viral illnesses and optimizing the fish's general growth parameters (Bharathi *et al.*, 2019). While there is growing public concern about antibiotic application in aquaculture due to the increased danger of the discovery of antibiotic-resistant bacteria in these conditions, this is a problem that affects not only aquaculture but also consumers, terrestrial animals, the environment, and other living things (Ghiassi *et al.*, 2021).

Prebiotics were added in response to the demand for safe food around the world as organic alternatives to antibiotic therapy as growth promoters, which increased fish resistance and decreased pathogen-related mortality rates (Ahmed *et al.*, 2015).

#### **Growth performance**

Organic selenium in the form of selenoyeast or selenomethionine may have advantages over its inorganic counterparts (Mahan, 1999). In the existing study, all investigated growth parameters were raised in all prebiotic-supplemented groups with the greatest



**Fig. 10.** Photomicrograph of H&E stained panel of spleen of *O. niloticus* pre-challenge (A) and post-challenge with *A. hydrophila* fed on ration contained 0 (B), 0.5 (C), 1 (D), and 2 mg (E) of Sel-Plex®1000 showing mixed red (R) and white (W) pulp, melanomacrophage centers (MMC), area of necrosis (N), and edema (E).

values in the T3 group (fed on Sel-Plex® 1 g/kg); these findings may be due to the fish's improved feed utilization. The obtained growth parameters findings are in accordance with those reported previously; in tilapia (Abdel-Tawwab and Mohapatra, 2008), African catfish (Abdel-Tawwab *et al.*, 2007a), channel catfish (Gatlin and Wilson, 1984), European seabass (*Dicentrarchus labrax*) (Abd El-Kader *et al.*, 2020) and grouper (Lin and Shiau, 2005) but with un-similar doses of organic selenium. However, the current results conflict with those investigated by Monteiro *et al.* (2007) who discovered that diets enhanced with organic or inorganic sources of selenium from 1 to 2 mg/kg<sup>-1</sup> levels had no effect on the growth of fish species. Variations between organic selenium doses used in these studies may be attributed to a variety of selenium origin and selenium concentration in the rearing water.

The present prebiotic may have had a positive impact on the feed conversion rate improvement by reducing the quantity of feed required to yield one unit of fish, which in turn decreased production costs. The findings concur with those of certain authors (Abdel-Tawwab and Wafeek, 2008).

#### **Hematological parameters**

Blood biochemical measurements are frequently used to detect potential changes in the fishes' general health state when they consume functional feed

additives (Dawood *et al.*, 2019; Moustafa *et al.*, 2021). Regarding the hematological parameters, there was a substantial increase in Hb, RBCs, MCV, and MCH in all supplemented groups, with a dramatic increase of PCV in the T3 and T4 groups. Moreover, a considerable elevation in WBCs, heterophil, eosinophil, and monocyte (%) among treated groups. These increased levels confirm that this rise was the main contributor to the improved growth in treated groups and this clearly indicates that the supplemented fishes were luxuriously endowed with defensive mechanisms presenting them as equipped conquerors against the impending potential pathogenic invasions or any disease as a result of Sel-Plex feed supplementation. These investigated results concur with those reported by previous authors (Ani *et al.*, 2009; Iqbal *et al.*, 2017). However, this is in contrast to El-Hammady *et al.* (2007). The hematological parameter results may differ based on the prebiotic kind and dosage, fish species, age, size, physiological status, and eating habits as well as the ecological circumstances (Osuigwe *et al.*, 2005).

#### **Biochemical analysis**

Investigating blood chemistry analyses mostly supplies important and vital information supporting the management of farmed fish and the diagnosis of their health status (Řehulka *et al.*, 2004). The noticeable decrease in ALT levels in the T4 prebiotic-supplemented group is a similar result to that recorded by Yařinkaya *et al.* (2008). This could be clarified by the proof that when liver cells are injured or their membranes are breached, allowing these enzymes to seep out, both ALT and AST levels should rise in the plasma (Abdel-Tawwab *et al.*, 2007a; Kouba *et al.*, 2014). However, there was a decrease in uric acid and AST levels in fish-fed Sel-Plex; the results coincided with Ani *et al.* (2009) and are in contrast to some researchers (Abdel-Tawwab *et al.*, 2007b; Abdel-Tawwab and Wafeek, 2008). Schaperclaus *et al.* (1992) clarified that albumin, globulin, and total protein measurement in plasma or serum is a valuable screening tool in fish, as it reflects the overall nutrition state. The significantly increased level of albumin, globulin, and total proteins in all prebiotic-supplemented groups; prebiotic supplementation may reflect the increased inherited body defense and better innate response of fish due to the use of prebiotic supplements (Andrews *et al.*, 2009). These variables were applied as a general symptomatic sign of fish health, immunological capability, stress, and nutritional condition of the fish. The outcomes concur with those that certain authors have reported (Ani *et al.*, 2009; Moustafa *et al.*, 2021); however, as opposed to those stated previously (Abdel-Tawwab *et al.*, 2007a, 2007b; Abdel-Tawwab and Wafeek, 2008).

#### **Immunological and antioxidant parameters**

The body defense of fish is significantly influenced by nonspecific immunity (Reda *et al.*, 2018). The ability of lysozyme to break down the bond between N-acetyl glucosamine and N-acetyl intracytoic acid

and so damage the pathogen microorganisms' cell wall structure makes it a crucial protective component of the humoral immune reaction (Han *et al.*, 2015). According to a recent study, all groups' immunological responses to *O. niloticus* fingerlings were fundamentally altered and improved, with the T3 group showing the greatest levels.

This study demonstrated that lysozyme is one of the most physiologically secure mechanisms in fish. It comes from neutrophils and macrophages that produce bacteriolytic substances into mucus and blood to help organisms fight off bacterial, viral, and parasitic diseases (Yano, 1997). One of the main immunological reactions in charge of the ability to directly kill harmful bacteria is bactericidal activity (Guardiola *et al.*, 2014). Lysozyme, whose concentration and activity in serum and skin mucus vary on fish species, ambient conditions, and dietary status, could be used to characterize it (Fast *et al.*, 2002). To boost the lysozyme activity of fish and other animals, which in turn improves the animal's immunological condition, B-lymphocyte generation is crucial (Kumar *et al.*, 2018).

The current findings clarified that Sel-Plex<sup>®</sup> supplementation substantially altered the dimensions of lysozyme action. Fish<sup>®</sup> that were encouraged to eat diets with 0.1% Sel-Plex diet showed the highest serum lysozyme activity. The immune-stimulating properties of dietary Sel-Plex<sup>®</sup> may be the cause of the increased lysozyme action (Dawood *et al.*, 2018b). The results coincided with those reported in goldfish (*Carassius auratus*) (Choi *et al.*, 2013), juvenile olive flounder (*Paralichthys olivaceus*) (Lee *et al.*, 2009), yellowtail kingfish (*Seriola lalandi*) (Le *et al.*, 2013), and juvenile *Tor putitora* (Khan *et al.*, 2016). Oxidative stress is brought on by an excess of reactive oxygen species (ROS) production (Sinha *et al.*, 2015). Oxidative stress can result from several unfavorable rearing circumstances, including crowding, high ammonia nitrogen levels, and low oxygen levels. This stress can induce a multitude of harms, including cell death through apoptosis or necrosis (Klein and Ackerman, 2003). A principal consequence of lipid peroxidation brought on by ROS is MDA (Xu *et al.*, 2010). The primary enzyme defense system against the negative effects of ROS was generated by the enzymes SOD, CAT, and GSH-Px (Zhou *et al.*, 2020). Fish's capacity for antioxidants can be determined by analyzing these variables.

It is notable that selenium assumes a significant part in actuating cell reinforcement guard frameworks including SOD, CAT, and GPx (Han *et al.*, 2011; Le and Fotedar, 2014). The past emitted catalysts are fundamental for rummaging the ROS (Atencio *et al.*, 2009). The emotional expansion in antioxidant activity (SOD, CAT, and GPx exercises) in all Sel-Plex<sup>®</sup> treated bunches with the most noteworthy expansion in the T3 bunch explain the oxidative ability of receptive oxygen species (ROS), which are conveyed by phagocytic

cells that have been enacted and are answerable for killing or corrupting immersed materials, including microorganisms (Atencio *et al.*, 2009).

Selenium is associated with the development of a few kinds of selenoproteins, among which GSH-Px is the main part of the invulnerable arrangement of the body (Rotruck *et al.*, 1973). By promoting the growth of selenium-enzymes and selenoproteins (selenoprotein P, thioredoxin reductase, and GPx framework), it protects the cells from oxidative materials. Hence, a higher concentration of these proteins will function more defensively, enhancing fish and other organisms' resistance (Kumar *et al.*, 2018), while low groupings of selenoproteins increment oxidative pressure, which then, at that point, prompts viral contamination (Sritunyalucksana *et al.*, 2011). Such selenoproteins partake effectively in the cell reinforcement protection framework and forestall the destructive impacts of receptive oxygen species (Tapiero *et al.*, 2003). They give security to cell films against oxidative pressure by utilizing decreased glutathione, which catalyzes the decrease of hydroperoxides and peroxides (Rider, 2009). The outcomes of the recent investigation are predictable with various other explorations (Abdel-Tawwab *et al.*, 2008; Khan *et al.*, 2016).

The prebiotic-enhanced T3 bunches in the ongoing review showed higher CAT activity levels with diminished MDA. The finding proposed that CAT and MDA were not really conversely related. This information recommends that supplementation of 1 mg Sel-Plex kg<sup>-1</sup> is by all accounts more powerful in reinforcing the antioxidant system prevention agent framework against oxidative pressure. These results are predictable with various other explorations (Abdel-Tawwab *et al.*, 2007a; Lin and Shiau, 2007; Dawood *et al.*, 2017, 2018b, 2020d). Since CAT is basically one of numerous cell reinforcement catalysts that could stop the blend of MDA, the clarification is fairly straightforward. The last MDA level of fish might be influenced by various cancer prevention agents other than proteins, such as nonenzymatic mixtures (such as L-ascorbic acid and E) and certain prebiotics and probiotics (Wang *et al.*, 2017).

#### **Antioxidant activity after experimental challenge with *A. hydrophila***

Prebiotics in the feeding routine have been demonstrated in various examinations to fundamentally increment fish protection from destructive microorganisms (Zhang *et al.*, 2020). Fish might encounter oxidative pressure due to *A. hydrophila*, one of the most common pathogenic microscopic organisms linked to aquatic environments (Turutoglu *et al.*, 2012). In the current study, Nile tilapia fingerlings fed Sel-Plex<sup>®</sup> revealed the highest SOD, CAT, and GPx activities were recorded in the prebiotic supplemented T3 group while, recorded sharp decline in MDA activity after infection with *A. hydrophila*. As investigated in the present study, *A. hydrophila* infections are described by the enactment

of solid ROS age in the host, which causes oxidative harm (Li *et al.*, 2013; Mir *et al.*, 2017). MDA is known as an indication of ROS-prompted cell harm, and the antioxidant system framework can diminish this harm (Dawood *et al.*, 2018b). The recent findings exhibited that adding a reasonable measure of Sel-Plex<sup>®</sup> to a feeding regimen smoothers unnecessary ROS age and brings down MDA levels. The outcome is comparable to studies by Rathore *et al.* (2020) which found that Sel-Plex<sup>®</sup> could increase Nile tilapia resistance to *A. hydrophila*, Chiu *et al.* (2010), which found that selenium could improve *M. rosenbergii* resistance, and Rudy and Fotedar (2013) increase marron, *Cherax cainii* resistance to *Vibrio mimicus*. *Aeromonas hydrophila* challenge was recorded 100% in all treated groups. The recent outcomes demonstrated that the fuse of Sel-Plex<sup>®</sup> in diet could move along opposition against *A. hydrophila* disease. The results concur with Rathore *et al.* (2020).

#### **Relative gene expression**

Levels of IL-1 $\beta$ , *TNF- $\alpha$* , and *SOD* gene expression are correlated with fish physiological status (Dinarello, 2018), oxidative status (E'cimovi'c *et al.*, 2018), and immune system health of the fish (Elbahnaswy and Elshopekey, 2020). IL-1 $\beta$ , and *TNF- $\alpha$* , the pro-inflammatory cytokine, had their gene expression levels enhanced in the Sel-Plex<sup>®</sup> supplemented groups in comparison to the control fish bunch. Simultaneously, those for insulin-like IGF-1, GHR, SOD, and GSH-Px were also improved at the same period. Furthermore, there was no massive distinction somewhere in the range of T3 and T4 bunches enhanced with 1 and 2 mg/kg Sel-Plex<sup>®</sup>, before and post challenge with *A. hydrophila*, with respect to the expression of IL-1 $\beta$ , and *TNF- $\alpha$*  genes in the liver. The outcomes are like that announced in previous research (Liu *et al.*, 2005; Cao *et al.*, 2011). Choi *et al.* (2013) and Khan *et al.* (2016) likewise noticed a critical connection between the development of chemical level and selenium healthful status and affirmed the idea that supplementing with selenium has a fundamental impact ( $p < 0.05$ ) on fish growth performance and serum growth hormone levels. Moreover, these outcomes sufficiently upheld the likely impact of Sel-Plex<sup>®</sup> to improve the resistance and anti-inflammation brought about by *A. hydrophila* disease in Nile tilapia.

#### **Histopathological findings**

Morphological respectability is fundamental for keeping up with the ordinary elements of the digestive tract (Gao *et al.*, 2013). Fish having a healthy gut have specific intestinal morphological boundaries (Han *et al.*, 2015). While contrasting different feeding regimes for aquatic animals, a significant morphometry marker to consider is the evaluation of intestinal health, including the ability to absorb nutrients and digestive functions through the length of intestinal villi, thickness of the muscular layer, and number of goblet cells (Yukgehnash *et al.*, 2020). Prebiotics in the eating routine can assist

cultivated fish with consuming their feed all the more successfully and keep up with the strength of their intestinal mucosal epithelium (Pelaseyed *et al.*, 2014). This was likewise affirmed through the upregulation of the growth hormone (GHR). In the recent study, Sel-Plex® considerably ameliorated each of the three intestinal measures (villus width, villus length, and crypt depth), representing the best overall intestinal structure-improving effect. The results are coincided with Ghaniem *et al.* (2022).

According to a histological investigation conducted mostly on Nile tilapia in the control group that was not given Sel-Plex® treatment, the infection with *A. hydrophila* caused noticeable degenerative alterations in the gut, hepatopancreas, spleen, and posterior kidney. The increase of the inflammatory genes TNF- and IL-1 (interleukin 1) further supports this inflammation. However, with a higher concentration of Sel-Plex®, the severity of the lesion was significantly reduced and showed clear improvement. Improved intestinal absorptive and defensive activities may be related to increased surface area available for absorption and/or produced mucus from many goblet cells, which has a bactericidal effect by covering pathogen receptors and protecting the integrity of the intestinal epithelium (Smirnov *et al.*, 2005; Iqbal *et al.*, 2020).

### Conclusion

Our knowledge of the functional nutritional techniques for using organic selenium (Sel-Plex®) as a prebiotic in freshwater fish diets has been advanced by recent investigation. It is possible to draw the conclusion that prebiotics boosts the antioxidant capacity of fish and raise disease resistance, which is important in modern intensive aquaculture. The dietary Sel-Plex® supplementation is used to help moderate the increased oxidative stress induced by *A. hydrophila*; they have the potential to be used as a prophylactic additive for Nile tilapia instead of antibiotics when pathogen challenges are present. Due to the improved findings on growth rate, physiological response, immunological reaction, and intestinal absorptive capacity, it may be advised to enrich the diets of Nile tilapia fingerlings with 1–2 g.kg<sup>-1</sup> of Sel-Plex®.

### Author contributions

The current work's purpose and design were contributed by all of the authors. The study's conception and design were created by Eman Moustafa and Amira Omar. Eman Moustafa, Amira Omar, Mohamed Zayed, and Wesam Abd ElAziz handled the preparation of the materials, gathering and analysis of the data. Wesam Abd ElAziz conducted the statistical analysis. The analysis of gene expression was performed by Mustafa Shukry. The histological investigation was conducted by Foad Farrag. Eman Moustafa, Amira Omar, Mohamed Zayed, and Wesam Abd ElAziz wrote the first draft of the work. All authors provided feedback on earlier drafts of the

work. Eman Moustafa then reviewed and corrected the final draft. The final version of the manuscript was read and approved by all authors.

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### Data availability

The current manuscript contains all the data supporting the findings of this study. Upon a reasonable request, the corresponding author will provide any further information needed.

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