Aquaculture International
https://doi.org/10.1007/s10499-024-01526-z

RESEARCH

Strengthening growth, digestion, body composition, haemato-biochemical indices, gene expression, and resistance to *Fusarium oxysporum* infection and histological structure in *Oreochromis niloticus* by using fructooligosaccharides and β-1,3 glucan mixture

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Received: 9 March 2024 / Accepted: 18 April 2024

Abstract
Prebiotics are fibers that promote beneficial gut bacteria and play a pivotal role in enhancing host health. This study delves into the impact of various levels of prebiotics, specifically fructooligosaccharides and β-1,3 glucan (Fβ), on the growth performance, biochemical, hematological parameters, gene expression, histological variations in the internal organs, and disease resistance to *Fusarium oxysporum* in tilapia (*Oreochromis niloticus*). Two hundred forty Nile tilapia, initially weighing 34.0 ± 0.1 g, were distributed into four groups and given a commercial diet with varying Fβ treatments: control (0 g/kg) (C), T1 (0.5 g/kg), T2 (1.0 g/kg), and T3 (1.5 g/kg) for 70 days. The study revealed significant improvement of the biochemical, hematological, and digestive enzyme activities, as well as histological changes in hepatopancreatic, intestine, and spleen sections with the use of Fβ. Moreover, the expression of innate humoral genes significantly increased ($P < 0.05$) in tilapia at the 1.5 g/kg Fβ group compared to the control. Notably, challenging with *F. oxysporum* exhibited lower mortality rates in the three treatments supplemented with additive prebiotics ($P < 0.05$). Consequently, the feed additives utilized in this study emerge as a viable alternative to enhance growth performance, biochemical and hematological parameters, gene expression, histological variations in internal organs, and disease resistance in fish farming on a large scale.

Keywords Fish status · Fructooligosaccharides · *Fusarium oxysporum* · β-1,3 glucan · *Oreochromis niloticus*

Introduction

Aquaculture emerges as one of the most promising solutions to address global food shortages, providing a substantial source of dietary protein derived from aquatic animals (Idenyi et al. 2022; Mohan et al. 2022; Eissa et al. 2022a). Among the numerous fish species

Handling Editor: Brian Austin

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cultivated, tilapia stands out as one of the most significant fish species in terms of volume produced. Widely distributed across more than 100 countries, Egypt ranks among the top three nations, producing 875 thousand tonnes (Abdel-Latif et al. 2020; Eissa et al. 2023d; Hendam et al. 2023). According to the FAO Food and Agricultural Organization (2022), the global tilapia harvest from aquaculture was 4.4 million tonnes in 2020. The term "tilapia" serves as a general descriptor for various fish species, primarily those belonging to the genera *Oreochromis* and *Sarotherodon*. The popularity of this species can be attributed to several key traits, including its adaptability, resilience against various diseases, and proficiency in thriving under challenging farming conditions (Fishstat 2015).

Nile tilapia is known for its resilience, and the occurrence of infection outbreaks remains a significant challenge in tilapia production, particularly in intensive farming conditions (El-Sayed 2019). Fish infections often stem from primary stressful events, with numerous pathogens commonly identified in instances of pathogenic infection (Roon et al. 2015; Zhang et al. 2015; Kotob et al. 2017).

Nutritional supplements play a prominent role in increasing health status through increasing fish immunity against disease infection (Alagawany et al. 2016; Khafaga et al. 2019; El-Sayed et al. 2021; Abd El-Hack et al. 2022; Makled et al. 2022; Sayed et al. 2023; El-Sayed et al. 2023).

β-Glucans and fructooligosaccharides are two primary prebiotics frequently selected to complement probiotics in fish farming (Song et al. 2014; Huynh et al. 2017; Eissa et al. 2024). Extensive scholarly investigations have elucidated the positive impact of both compounds on the gut microbiome, unequivocally confirming their roles as growth stimulants within the fish farming sector (Mohammadian et al. 2019). The underlying mechanism of prebiotics involves the breakdown of these substances into their respective sugars within the fish's digestive tract. Subsequently, these sugars serve as carbon sources for beneficial bacteria (Goh and Klaenhammer 2015). Beyond their role as growth stimulants, prebiotics have demonstrated the potential to enhance development productivity, fortify immune system function, and bolster resistance against diseases in fish aquaculture. Additionally, they play a crucial role in preventing the transmission of pathogenic diseases (Eissa et al. 2022b; Eissa et al. 2023d, Eissa et al. 2024).

To evaluate both the structure and physiological conditions of fish challenged to multiple toxins, blood test results have been extensively utilized as pathological markers (Panjvini et al. 2016; Baloch et al. 2022). The identification of specific organs and the overall health status of animals are significantly enhanced through the analysis of serum biochemical parameters. This approach is particularly valuable for its capability to signal various alterations in stressful conditions, as recommended by Usman et al. (2023). Histopathological methods are instrumental in assessing the sub-lethal effects of viruses or contaminants, with physiological changes serving as indicative markers. Moreover, structural alterations offer a swift and intermediate means of identifying stressors and evaluating their impact on tissues. (Figueiredo-Fernandes et al. 2007).

Various species of *Fusarium* are commonly found in ecosystems (Michielse and Rep 2009; Eissa et al. 2023a). *Fusarium* infections have been reported in amphibians and several aquatic animals, as reported by Salter et al. (2012), Eissa et al. (2023b), and Jastaniah et al. (2023). Fish experiencing fungal issues often exhibit concurrent health issues, such as microbial infections or fluctuations in water acidity or humidity. In addition to various aquatic fungi, *Saprolegnia* species pose challenges for both wild and cultivated freshwater fish. *Aphanomyces* and *Fusarium* are also recognized as significant contributors to fish diseases affecting both aquatic animals and crustaceans (Hatai 2012). Fish afflicted with microbiological infectious diseases not only manifest visible morphological changes and alterations but also experience

substantial alterations in hemoglobin levels and hepatic enzyme function (Kulatunga et al. 2017; Ukwe and Oladapo-Akinfolarin 2019; Eissa et al. 2023c).

The objective of this study was to evaluate the possible application of Fβ at different levels in the diet of *Oreochromis niloticus* and to elucidate its effects on weight, body composition, histological changes, gene expression, and resistance against *F. oxysporum* infection.

Materials and methods

Setup and preparations for the study

The study was conducted during the period of August 2023 to November 2023 at a privately owned aquaculture farm in Egypt, utilizing 12 concrete ponds, each measuring $1 \times 1 \times 1.20$ m with a water volume of 1 m^3. The experiment involved four feed treatments: 0.0 g/kg Fβ, 0.5 g/kg Fβ, 1.0 g/kg Fβ, and 1.5 g/kg Fβ. The concrete ponds maintain a daily water change rate of 30%.

Management of experimental fish

Two hundred and forty normal *O. niloticus* were obtained from a commercial fish farm, with an initial body weight of 34.0 ± 0.1 g/fish. The fish were uniform in their body weights and in good health, as indicated by their regular eating habits and active surface swimming during feeding times. Fish were fed a commercial diet (30% protein) during acclimatization period of 15 days before the commencement of the trials. Twenty fish per m^3/pond of tilapia were stocked, with each pond equipped with constant aeration. The feeding trial was conducted for 70 days.

Experimental feed management

The experimental fish diets comprised 30.22% crude protein, 8.16% crude lipid, and 7.22% ash. Fish were fed three times a day, and the feeding rate varied based on their biomass, maintaining it at 2.5%. The proximate composition of the ingredients and experimental diets is as outlined in Table 1. The formulation of the experimental diets was meticulously calculated to meet the optimal nutrient requirements for Nile tilapia (*O. niloticus*) requirement (NRC 2011).

Assessment of water quality

The assessment of water quality involved daily measurements of key indicators at 3 p.m., encompassing pH, temperature, dissolved oxygen, salinity, and ammonia. An Ecosenset DO330M MultiMeter (YSI, Brannum Lane, OH, USA) was utilized for measuring salinity, pH, and dissolved oxygen in the water. Ammonia (NH_3) was measured using HI 34715-12A instrument (Table 2).

Growth performance

Randomly, sampling was carried out at fortnight intervals, and the final data were recorded for the calculation of growth efficiency variables, including final body weight (FW), weight

Table 1 The formulation and proximate composition of the experiment diets (g/kg)

Ingredient	C	T1	T2	T3
Fish meal (CP 72%)	110.0	110.0	110.0	110.0
Soybean meal (CP 48%)	360.0	360.0	360.0	360.0
Rice bran	200.0	200.0	200.0	200.0
Wheat bran	200.0	200.0	200.0	200.0
Yellow corn	60.0	59.5	59.0	58.5
Fructooligosaccharides and β-1,3 glucan (Fβ)	0	0.5	1.0	1.5
Fish oil	15.0	15.0	15.0	15.0
Soybean oil	15.0	15.0	15.0	15.0
Molasses	20.0	20.0	20.0	20.0
Di-calcium phosphate	10.0	10.0	10.0	10.0
Vitamin & minerals + premix*	10.0	10.0	10.0	10.0
Proximate composition (% dry weight basis)				
Dry matter (DM)	91.40	91.42	91.50	91.47
Crude protein (CP)	30.22	30.26	30.29	30.27
Crude fat	8.16	8.17	8.16	8.16
Ash	7.22	7.22	7.23	7.21
Crude fiber	6.61	6.60	6.59	6.60
Nitrogen-free extract	46.7	46.6	46.7	46.7
Gross energy (kcal/g)[3]	4.70	4.70	4.71	4.70

*Vitamins and mineral premix: 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g; and Co, 4.8 mg

gain (WG), average daily gain (ADG), specific growth rate (SGR), feed intake, and survival rate.

The calculation was performed using the following equations:

$$\text{Weight gain (g/fish) is calculated as WG} = \text{Wt} - \text{W0},$$

where Wt is its final mean weight in (g) and W0 is the fish's beginning mean weight in (g).

$$\text{Average daily gain (ADG)} = \text{Wt} - \text{W0}/n,$$

where W0 and Wt are the starting and final mean weights of fish in grams, respectively, and n is the length of the period. This yields the mean daily gain (g/fish/day).

$$\text{SGR} = 100 \times [(\ln\text{Wt} - \ln\text{W0})/\text{days}] \text{ is the particular growth rate (\%)/day},$$

where W0 and Wt are the fish's beginning and final average weights in (g), respectively.

$$\text{Fish survival rate (\%)} = 100 \times (\text{final fish count} \,/\, \text{starting fish count}).$$

Table 2 Water quality parameter during experimental period

Temperature (°C)	26.70 ± 0.29
Salinity (ppt)	2.03 ± 0.03
pH	7.97 ± 0.07
DO (mg/L)	5.94 ± 0.01
NH_3 (ppm)	0.34 ± 0.00

Feed utilization

The feed utilization was calculated using the following formula:

$$\text{Feed intake (g/fish)} : \text{the quantity of fish feed given to the fingerlings during the experimental period (g).}$$

$$\text{Feed conversion ration (FCR)} = \text{feed intake (g)/weight gain (g)}$$

Chemical analysis of fish body and feed

The proximate composition of fish tissue was analyzed including moisture content, crude protein, crude lipid, and ash levels. Ten fish and feed samples were collected randomly at the beginning and at the end of each experiment. Ten fish were chosen randomly on the loading day for body chemical analysis. The evaluation of moisture, crude protein, ether extract, and ash levels in the entire fish carcasses was conducted on a dry matter basis, following the methods outlined by AOAC Association of Official Analytical Chemists (1997).

Evaluationof blood samples

Blood samples were drawn from the distal vertebral vein in five randomly selected fish specimens by using heparinized syringes following the methods described by Feldman et al. (2000). Hemoglobin levels were assessed using Drabkin's solution, the Natt-Herrick solution, and the cyanomet hemoglobin technique, along with the counting of erythrocytes and leukocyte numbers using a hemocytometer, as outlined by Stoskopf (1993). Data were collected using PCV% and differential count of leukocytes in accordance with the methods outlined by Dacie and Lewis (1991). Additionally, the differential leukocyte count (DLC) was calculated using the formula provided by Thrall (2004): total leukocyte count / 100 × the number of each white cell, yielding the absolute DLC. The formula for blood performance (BP) was calculated as Ln (Hb (g/dL) + Ln Ht (%) + Ln RBC (*105/mm^3) + Ln WBC (*103/mm^3) + Ln TP (g/L), as specified by Esmaeili (2021).

Biochemicalanalysis

The amount of globulins in the blood, total proteins, albumin, and creatinine was measured at 540 nm and 550 nm, respectively, with $n = 5$ samples per group (Doymas et al. 1981; Dumas and Biggs 1972). Colorimetric measurements of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activity were conducted with $n = 5$ samples per group at 540 nm, following the methods by Reitman and Frankel (1957). Cholesterol and triglycerides were measured using commercial clinical kit procedures from Bio-Merieux, France (Fynn-Aikins et al. 1992). Glucose levels were measured using kits from Bio-Merieux, France, in accordance with Trinder's method (1969). The activity of amylase and lipase was tested in triplicates using Bio-Merieux kits (Diab et al. 2023). Red blood cells (RBCs), hemoglobin, and white blood cells (WBCs) were measured based on the method described by Makled et al. (2017).

Oxidative stress and immune-marker levels

Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), lysozyme (LYZ), and immunoglobulin M (IGM) activities were quantified utilizing an ELISA kit from Thermo Fisher, USA, following the manufacturer's protocol (Abdel-Tawwab et al. 2018; Hao et al. 2020).

Testing gene expression

Extraction of genetic material (DNA)

Total RNA was extracted from 50 mg of liver tissues ($n=5$ samples per group) using the Trizol reagent (Invitrogen, Thermo Fisher, USA) following the manufacturer's instructions. The RNA concentration was validated using a Nano-drop (Uv–Vis) spectrophotometer 1612 from Milton Roy, Tokyo, Japan. Complementary DNA (cDNA) was synthesized using the cDNA production kit from Thermo Fisher, USA, according to the supplier's directions. Subsequently, the cDNA specimens were stored at − 20 °C until further use.

Real time qPCR (RT-PCR)

The specific primer sequences, product sizes, and NCBI GenBank accession numbers for the genes producing tumor necrosis factor-alpha (*TNF-α*), interleukin one beta (*IL-1β*), interleukin eight (*IL-8*), growth hormone (*GH*), and insulin-like growth factor (*IGF-1*) are listed in Table 3. Additionally, the housekeeping gene beta-actin was utilized as a reference for assessing mRNA expressions (Table 3). RT-PCR, to measure the mRNA expression folds of the target genes, was performed using the Thermo Fisher SYBR kit. The thermocycling parameters included an initial step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 120 s, 60 °C for 1 min, and 72 °C for 20 s. The mRNA expression folds of each target gene were normalized and standardized to β-actin mRNA transcripts using the $2^{-\Delta\Delta CT}$ method as described by Schmittgen and Livak (2008).

Challenge testing for *Fusarium oxysporum*

Fusarium oxysporum isolated from *O. niloticus* was provided by the Microbiological Division of the Fish Infections, Department at the Veterinary Institute, Dokki, Giza, Egypt. Following the procedures outlined by Munir et al. (2018), *F. oxysporum* was cultured on Sabouraud dextrose agar (SDA, Thermo Fisher, USA) supplemented with penicillin (100 UI/mL) and streptomycin (100 µg/mL) to generate spore suspensions. The cultures were then incubated at 26 °C for 7 days. To collect conidial mass, each plate was filled with 25.0 mL of sterile distilled water, and the suspension was collected in sterile tubes, passing through a pair of layers of sterile gauze as filters. The conidial concentration in the suspension was determined using a hemocytometer's erythrocyte counting chamber and adjusted to 4.0×10^3 conidia/mL in sterile distilled water.

Subsequently, twenty surviving fish from each group were injected with *F. oxysporum* (4.0×10^3 conidia/mL) using a sterile needle, and their condition was monitored daily for the next 15 days. Another twenty fish, also injected, received a baseline diet. The cumulative death percentage was calculated based on the outcomes of the challenge test.

Table 3 The sequence of forward and reverse primers used in this study

Gene	Primer sequence	GenBank accession no	Size	Slope	Efficiency
TNF-α	F:AAGCCAAGGCAGCCATCCAT R:TTGACCATTCCTCCACTCCAGA	NM_001279533.1	184 bp	−3.32	100.08
Il-8	F:CTGTGAAGGCATGGGTGTGGAG R:TCGCAGTGGGAGTTGGGAAGAA	NM_001279704.1	111 bp	−3.40	96.80
Il-1β	F:CAAGGATGACGACAAGCC AACCR: AGCGGACAGACATGA GAGTGC	XM_019365844.2	149 bp	−3.37	96.7
GH	F:ACATCATCAGCCCGATCGAC R:TCAGCAGCAAGATTCCCGTT	XM_003442542.5	183 bp	−3.43	97.8
IGF-1	F:TTGTCTGTGGAGAGCGAGGCTT R:CAGCTTTGGAAGCAGCACTCGT	XM_019346352.2	103 bp	−3.39	95.5
B-actin	F:CAGCAAGCAGGAGTACGATGAG R: TGTGTGGTGTGTGGTTGTTTTG	XM_003455949.2	136 bp	−3.35	97.01

Histological examination

Histological sections of hepatopancreas, intestine, and spleen from 3 fish were cut at 6 μ in thickness by rotatory microtome (Leica RM2125, Germany) and were stained with hematoxylin and eosin (H&E) dyes and examined using a light microscope (Nikon CX53, Japan) equipped with an ocular attachment (Carl Zeiss ERc 5s, Germany) and software (Zeiss, Germany). The ImageJ program (NIH, USA) was employed for the evaluation of histological characteristics, following the methods outlined by Matrosova et al. (2021) and Nikiforov-Nikishin et al. (2022).

Statistical analysis

GraphPad Prism (V5 San Francisco, CA, USA) was employed for all statistical analyses. Before conducting any statistical analysis, the assumptions of homoscedasticity and normality were checked. Analysis of variance (ANOVA) was utilized to assess all variables under consideration, determining whether Fβ levels had a significant ($P<0.05$) impact on the observed outcomes. Additionally, a follow-up trend analysis using orthogonal polynomial analysis, as outlined by Wei et al. (2019), was performed to ascertain whether the effect was linear or quadratic. Differences between means were examined using Duncan's multiple range test. The results are presented as the standard error of the mean, or Mean ± SE.

Results

Assessment of growth performance and feed utilization

The survival rate (%) of the 0.5 g/kg Fβ, 1.0 g/kg Fβ, and 1.5 g/kg Fβ treatments surpassed that of the control group (84.67 ± 1.67%) with the values of 95.33 ± 1.67%, 100.00 ± 0.00%, and 98.33 ± 1.67%, respectively, as shown in Table 4. Additionally, a

Table 4 Variations in growth and feed utilization of Nile tilapia, *Oreochromis niloticus*, fed diets supplemented with different levels of Fβ for 70 days

Parameters	C	T1	T2	T3
Initial fish weight (g)	34.00 ± 0.06^{a}	34.00 ± 0.12^{a}	34.00 ± 0.06^{a}	34.10 ± 0.06^{a}
Final fish weight (g)	66.05 ± 1.00^{c}	72.35 ± 0.98^{b}	75.32 ± 0.88^{ab}	76.75 ± 0.97^{a}
Weight gain	32.05 ± 0.94^{c}	38.35 ± 0.87^{b}	41.32 ± 0.83^{ab}	42.65 ± 1.01^{a}
Weight gain (%)	0.95 ± 0.02^{c}	1.08 ± 0.02^{b}	1.14 ± 0.01^{a}	1.16 ± 0.02^{a}
Feed intake	51.00 ± 0.09^{a}	51.00 ± 0.17^{a}	51.00 ± 0.09^{a}	51.15 ± 0.09^{a}
Feed conversion ratio (FCR)	1.59 ± 0.04^{a}	1.33 ± 0.03^{b}	1.24 ± 0.02^{bc}	1.20 ± 0.03^{c}
Specific growth rate (SGR; %/fish/day)	0.95 ± 0.02^{c}	1.08 ± 0.01^{b}	1.14 ± 0.01^{a}	1.16 ± 0.02^{a}
ADG	0.46 ± 0.01^{c}	0.55 ± 0.01^{b}	0.59 ± 0.01^{ab}	0.61 ± 0.01^{a}
Initial number	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
Final number	19.33 ± 0.33^{a}	19.67 ± 0.33^{a}	20.00 ± 0.00^{a}	19.67 ± 0.33^{a}
Fish biomass per m^3	1276.36 ± 12.80^{c}	1422.19 ± 7.98^{b}	1506.47 ± 17.53^{a}	1509.51 ± 34.17^{a}
Survival rate (%)	84.67 ± 1.67^{a}	95.33 ± 1.67^{b}	100.00 ± 0.00^{c}	98.33 ± 1.67^{d}

[a, b, c] mean in a row without a common superscript letter were significantly different ($p < 0.05$). Values were means and standard errors

significant improvement was observed in the final body weight, weight gain, and SGR of Nile tilapia fed on the 0.5 g/kg Fβ, 1.0 g/kg Fβ, and 1.5 g/kg Fβ groups ($P < 0.05$). The 1.5 g/kg Fβ group exhibited the best FCR. Moreover, the fish biomass in the 0.5 g/kg Fβ, 1.0 g/kg Fβ, and 1.5 g/kg Fβ groups exceed that of the control group (1276.36 ± 12.80 g per m^3) with fish biomass recorded at 1422.19 ± 7.9 g, 1506.47 g, and 1509.51 ± 34.17 g per m^3, respectively.

Chemical composition of the entire body

Ten fish were selected for analysis on the dry matter, protein, and ash content of their entire bodies. In comparison to the control group, all Fβ additive groups demonstrated a significant enhancement ($P < 0.05$) in the tested parameters including the dry matter, protein, ether extract, and ash (%) as illustrated in Table 5.

Hematological and biochemistry parameters

The hematological parameter findings for each of the four treatment groups are presented in Table 6. Fish treated with Fβ exhibited significantly ($P < 0.05$) lower levels of ALT, AST, ALP, albumin, cholesterol, and triglycerides compared to the control group. Conversely, there were notably higher values of globulin and total protein, along with increased activity of digestive enzymes (lipase and amylase) and enhanced hematological indices ($P < 0.05$). Remarkably, the level of creatinine remained consistent across all treatments.

Biomarkers for immunity and oxidative markers

The immunological and antioxidant activities observed in different groups of Nile tilapia are listed in Table 7. Antioxidant enzymes, including SOD, CAT, and GPx, showed significantly higher levels ($P \leq 0.05$) in fish fed with Fβ compared to the control group,

Table 5 Proximate composition of Nile tilapia's, *Oreochromis niloticus*, whole body that fed diets supplemented with Fβ for for 70 days (% of dry basis)

Parameters	Initial	Final			
		Control	T1	T2	T3
Dry matter (%)	23.12 ± 0.13^{a}	24.26 ± 0.13^{b}	24.62 ± 0.05^{c}	24.74 ± 0.05^{d}	24.78 ± 0.03^{e}
Crude protein (%)	50.34 ± 0.04^{a}	53.34 ± 0.04^{b}	55.35 ± 0.85^{c}	57.35 ± 0.34^{d}	59.66 ± 0.30^{e}
Ether extract (%)	20.23 ± 0.11^{a}	20.63 ± 0.11^{b}	20.71 ± 0.33^{c}	20.70 ± 0.16^{d}	20.43 ± 0.14^{e}
Ash (%)	18.53 ± 0.03^{a}	14.53 ± 0.03^{a}	15.29 ± 0.13^{a}	15.42 ± 0.02^{a}	15.56 ± 0.20^{a}

[a, b, c] mean in a row without a common superscript letter were significantly different ($p < 0.05$). Values were means and standard errors

Table 6 Serum biochemical and hematological parameters of *Oreochromis niloticus* fed Fβ-supplemented diets for 70 days

Parameters	Control	T1	T2	T3
AST (U/L)	12.76 ± 0.25^{a}	10.69 ± 0.32^{b}	9.44 ± 0.29^{c}	9.01 ± 0.12^{c}
ALT (U/L)	41.20 ± 0.21^{a}	39.24 ± 0.26^{b}	38.57 ± 0.36^{b}	37.37 ± 0.23^{c}
ALP (U/L)	31.38 ± 0.37^{a}	30.53 ± 0.15^{b}	29.88 ± 0.27^{b}	28.79 ± 0.14^{c}
Albumin (g /dL)	2.14 ± 0.05^{d}	3.03 ± 0.10^{c}	3.45 ± 0.09^{b}	3.85 ± 0.10^{a}
Globulin (g /dL)	1.70 ± 0.05^{b}	1.86 ± 0.05^{ab}	1.93 ± 0.03^{a}	2.08 ± 0.10^{a}
Total protein (g /dL)	3.91 ± 0.08^{d}	4.98 ± 0.10^{c}	5.47 ± 0.15^{b}	5.97 ± 0.22^{a}
RBCs ($\times 10/mm^3$)	0.87 ± 0.04^{c}	1.17 ± 0.01^{b}	1.26 ± 0.02^{a}	1.34 ± 0.02^{a}
Hemoglobin (g/100 mL)	4.83 ± 0.10^{b}	5.20 ± 0.04^{a}	5.27 ± 0.03^{a}	5.29 ± 0.02^{a}
WBCs ($\times 10^3/mm^3$)	30.45 ± 0.70^{c}	32.24 ± 0.25^{b}	32.88 ± 0.52^{ab}	33.82 ± 0.19^{a}
Creatinine (mg /dL)	0.45 ± 0.82^{ab}	0.46 ± 0.01^{ab}	0.46 ± 0.01^{ab}	0.46 ± 0.02^{b}
Glucose (mg /dL)	13.52 ± 0.09^{a}	12.79 ± 0.16^{b}	13.34 ± 0.11^{a}	11.63 ± 0.07^{c}
Amylase (U/L)	12.46 ± 0.08^{d}	15.69 ± 0.15^{c}	16.76 ± 0.09^{b}	18.62 ± 0.09^{a}
Lipase (U/L)	24.52 ± 0.49^{c}	28.49 ± 0.46^{b}	30.91 ± 0.13^{a}	32.00 ± 0.16^{a}
Cholesterol (mg /dL)	78.82 ± 0.82^{a}	75.11 ± 0.33^{b}	74.08 ± 0.57^{b}	74.46 ± 0.57^{b}
Triglycerides (mg /dL)	116.20 ± 0.71^{b}	114.80 ± 0.25^{a}	113.01 ± 0.09^{a}	112.59 ± 0.10^{a}

[a, b, c, d] mean in a row without a common superscript letter were significantly different ($p < 0.05$). Values were means and standard errors

with the 1.5 g/kg Fβ (T3) group exhibiting the highest activity ($P < 0.05$). MDA levels were notably lower ($P < 0.05$) in all Fβ treatments than in the control group. Additionally, compared to the control group, the inclusion of Fβ in feed significantly increased ($P < 0.05$) IgM levels and stimulated serum LYZ activity across various treatments.

Gene expression outcomes

A pronounced upsurge in the mRNA expression levels of immunity-related cytokine genes (*TNF-α*, *IL-1β*, and *IL-8*) in proportion to the rising Fβ levels in the 0.5 g/kg Fβ, 1.0 g/kg Fβ, and 1.5 g/kg Fβ groups is compared to the control group. The relative mRNA expression of growth-correlated genes such as *GH* and *IGF-1* genes significantly

Table 7 The values of MDA, oxidative enzyme activities, LYZ, and IgM of *Oreochromis niloticus* fed diets supplemented with different levels of Fβ-supplemented diets for 70 days

Parameters	C	T1	T2	T3
MDA (nmol/dL)	2.11 ± 0.02^{a}	2.03 ± 0.02^{b}	1.91 ± 0.02^{c}	1.87 ± 0.02^{c}
SOD (IU/L)	12.43 ± 0.15^{d}	13.61 ± 0.08^{c}	14.24 ± 0.05^{b}	14.89 ± 0.24^{a}
CAT (IU/L)	11.41 ± 0.06^{d}	12.40 ± 0.05^{c}	12.87 ± 0.02^{b}	13.16 ± 0.10^{a}
GPx (IU/L)	20.64 ± 0.04^{d}	22.22 ± 0.20^{c}	23.19 ± 0.21^{b}	23.77 ± 0.05^{a}
LYZ (µg/mL)	1.42 ± 0.02^{c}	1.74 ± 0.07^{b}	1.94 ± 0.08^{ab}	2.11 ± 0.09^{a}
IgM (mg/mL)	21.42 ± 0.14^{d}	23.06 ± 0.12^{c}	24.17 ± 0.23^{b}	26.28 ± 0.22^{a}

[a, b, c, d] mean in a row without a common superscript letter were significantly different ($p < 0.05$). Values were means and standard errors

increased ($P < 0.05$) compared to the control group, with treatment 1.5 g/kg Fβ exhibiting the most robust growth-promoting effect in comparison to 0.5 g/kg Fβ and 1.0 g/kg Fβ levels (Fig. 1). The 1.5 g/kg Fβ treatment demonstrated the most potent immune-stimulant effect.

Challenge test using *Fusarium oxysporum*

On the third- and fourth-day post-challenge, the fish began to perish, exhibiting increased mucus discharges, scale separation, and hemorrhages in various areas of their external body. Postmortem examination revealed a pale, swollen liver with white nodules scattered throughout its outer layer and a distended gallbladder. The internal organs of the affected fish were utilized to re-isolate *F. oxysporum*.

In a dose-dependent manner, fish fed with any of the Fβ-supplemented treatments exhibited higher survival rates than those fed with the control treatment (Fig. 2). *O. niloticus* showed significant ($P < 0.05$) differences in survival rates between the treated groups and the control during the first and second weeks of the *F. oxysporum* challenge. Overall, fish fed with Fβ demonstrated superior survival rates in a dose-dependent manner across treatments compared to the control (Fig. 2).

Histopathological evaluation

Examination of hepatopancreatic sections

The liver maintained its treated histology throughout the entire set of trials in comparison to the infected control. Hepatocytes in the infected control group showed abnormalities in appearance caused by *F. oxysporum* infection (Fig. 3A), with easily recognizable nuclei, necrosis, and kupffer macrophage cells within enlarged sinusoid capillary, and central hepatic veins were engorged with blood. However, when utilizing prebiotic preparations based on Fβ (group T2), initial signs of treatment become apparent (Fig. 3B). The liver parenchyma's structure in the T2 and T3 groups closely adhered to the normal structures: improving the hepatocytes with proper nuclei, clearly visible central veins, endothelium, and sinusoidal blood were observed (Fig. 3C, D).

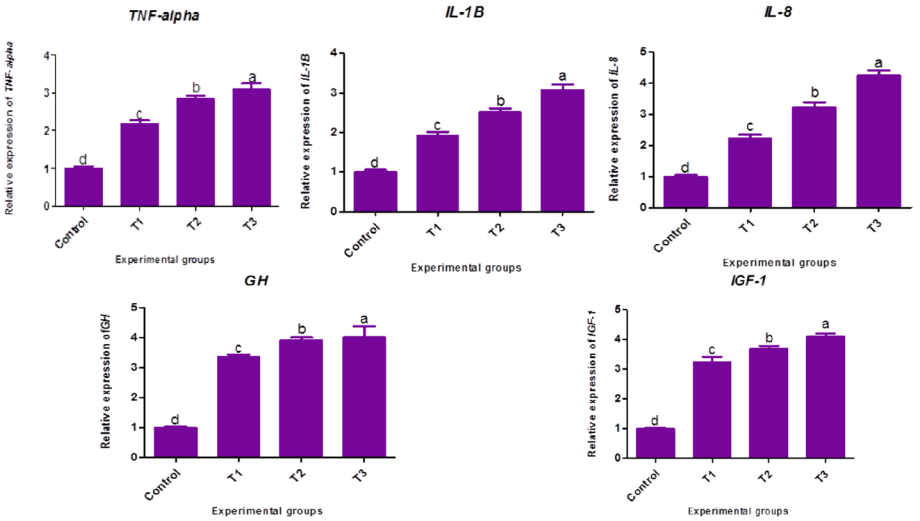

Fig. 1 Evaluation of immuno- and growth-related gene expression, including *TNF-alpha*, *IL-1beta*, *IL-8*, *GH*, and *IGF-1*, in control (C) and various treatments (T1, T2, and T3) (data are represented as means ±SD); control: 0.0 g/kg Fβ (C), 0.5 g/kg Fβ (T1), 1.0 g/kg Fβ (T2), and 1.5 g/kg Fβ (T3)

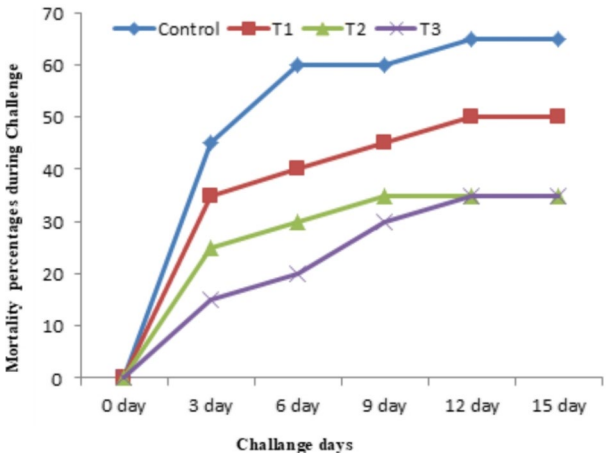

Fig. 2 Impact addition of Fβ with various levels in *O. niloticus* upon challenge with *F. oxysporum* infection

Examination of intestinal sections

The intestines maintained their typical histology throughout the entire series of trials in comparison to the control. After treatment of infected tilapia, an improvement in intestinal tissue structures was demonstrated (Fig. 4C, D). Treatments compared to infected control group (Fig. 4A) exhibited a significant increase in villi width and length ($P < 0.05$), and T3 was identified as the group with the longest and widest villi (Figs. 4 and 5).

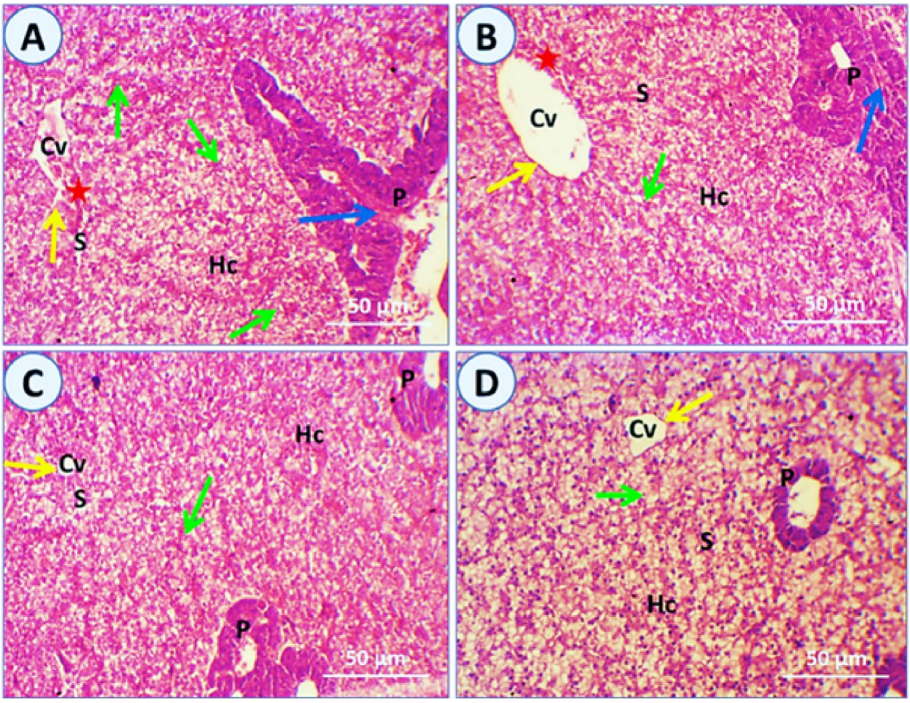

Fig. 3 Microscopic sections for hepatopancreatic *O. niloticus* after infection for the experimental groups (T0 group (infected control group)): (**A**), T1 group: (**B**), T2 group: (**C**), and T3 group: (**D**). Hepatocytes, hepatosinusoids (S), central vein (Cv), endothelium (Yellow arrows), firm adhesion of the leukocytes margination (Red stars), pancreas (P), inflammatory cells (Blue arrows), and Kupffer cells (Red stars). [H&E, Bar = 50 µm]

Examination of spleen sections

Spleen sections revealed some slight changes across the various tested groups. In all tested sections, the spleen sections displayed splenic red pulp, splenic white pulp, and the splenic capsule. Notably, lymphocyte colonies were discernible in both infected control group (Fig. 6A), and treatment groups (Fig. 6B–D).

Discussion

The Nile tilapia, *O. niloticus*, is highly recommended for its excellent meat quality, consumer appeal, and well-established rearing techniques, making it a valuable species for freshwater fish farming (Souza et al. 2019). However, infectious diseases and environmental stresses stand out as major and frequent challenges hindering the growth of the aquaculture industry (Baldissera et al. 2017; De Souza et al. 2017). Historically, the fish farming sector has heavily relied on chemotherapy treatments and antibiotics to combat infectious diseases. However, the overuse of these substances has raised concerns,

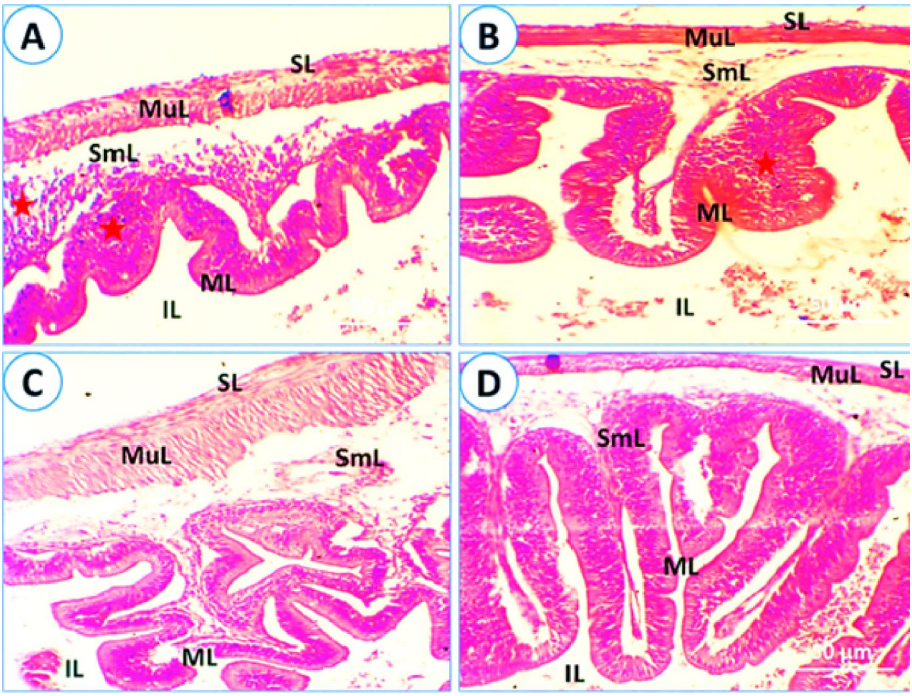

Fig. 4 Microscopic sections of intestine of *O. niloticus* after infection for the experimental groups (T0 group (infected control group): (**A**), T1 group: (**B**), T2 group: (**C**), and T3 group: (**D**). Intestinal lumen (IL), intestinal mucosa layer (ML), intestinal submucosa layer (SmL), intestinal muscular layer (MuL), and intestinal outer serosa layer (red stars). H&E, Bar = 50 µm

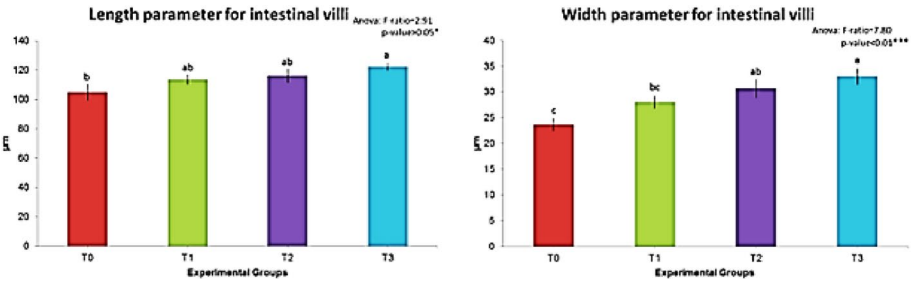

Fig. 5 Graph for intestinal parameters (length and width); each column showed the M ± SE for four current experimental groups ($n = 3$ for each group), and the different subscript letters (a, b, and c) above each column illustrated the highly significant width villi parameter and non-significant villi length parameter

prompting a shift toward incorporating probiotics, prebiotics, and synbiotics into aquaculture feed production (Fečkaninová et al. 2017; Morselli et al. 2019, 2020).

This study aimed to evaluate the effect of Fβ prebiotic supplementation in the feed of Nile tilapia and to examine the fish's tolerance to *F. oxysporum* infection. The effects of Fβ were evaluated across various parameters, encompassing water quality, growth performance, blood biochemical parameters, internal organ histology, and the expression of growth and immune-related genes. Good water quality is pivotal for successful

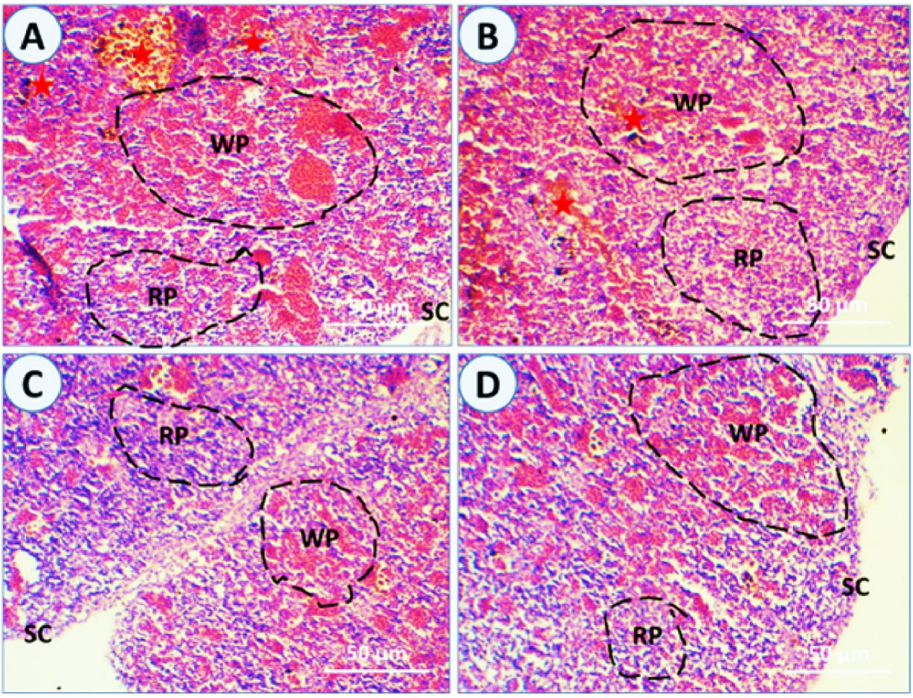

Fig. 6 Microscopic sections for spleen of *O. niloticus* after infection for the experimental groups (T0 group (infected control group) (**A**), T1 group (**B**), T2 group (**C**), and T3 group (**D**)). Splenic red pulp (RP), splenic white pulp (WP), and splenic capsule (SC) (H&E, bar = 50 μm)

aquaculture production, and a thorough comprehension of the interplay between feed components and aquatic conditions is essential for achieving optimal growth, survival, and overall production (Soundarapandian and Babu 2010).

The growth outcomes of fish supplemented with Fβ exhibited a noticeable increase in weight gain, growth rate, and survival rate when compared to the control group. Research by Ebrahimi et al. (2012) suggested that dietary immunogens as prebiotic supplementation in the range of 1 to 1.5 g/kg have the potential to enhance feed efficiency, developmental outcomes, and immunity to infection in fingerling *Cyprinus carpio*, as well as in our study, the better range was 1 to 1.5 g/kg Fβ. Additionally, Xu et al. (2022) found that applying prebiotics to younger fish could enhance their effectiveness, and the use of multiple oligosaccharide prebiotic components might result in a more significant improvement in growth.

In the current study, β-1,3 glucan (β-1,3 GF) demonstrated an improvement in Nile tilapia growth and feed consumption. Furthermore, it exhibited a significantly lower FCR and higher WG compared to the control. These findings align with previous research indicating that feeding β-glucans enhances development and feed consumption in various species such as red seabream (Dawood et al. 2017), tilapia (Pilarski et al. 2017), and Pacific white shrimp (Boonanuntanasarn et al. 2016; Li et al. 2019).

Similarly, *O. niloticus* fed with a synbiotic rich in glucans and fructooligosaccharides demonstrated a significant increase in weight gain and SGRs compared to non-treated fish

(Ismail et al. 2019). The growth-promoting effect of this molecule is believed to depend on its concentration, solubility, and structures, in addition to the species under investigation.

According to the present study's body composition examination, fish in the 1.5 g/kg Fβ group, in particular, exhibited significantly higher levels of crude protein and dry matter contents compared to fish in the other control and experiment groups after probiotics were added to their water. Ash content, on the other hand, improved the most in the 1.5 g/kg Fβ and control groups. These findings align with the work of Munir et al. (2016), who demonstrated that prebiotic-based diets have a more favorable impact on *C. striata* fingerlings development, body composition, feed utilization, and survival.

The current results revealed a substantial impact of using Fβ in the regulation of biochemical and hematological parameters, as well as digestive enzymes in treated groups relative to the control. This is consistent with Anguiano et al. (2013), who illustrated that rather than being attributed to an increase in the functioning of digestive enzymes, the reported gains in digestible nutrients in fish after receiving prebiotic treatment seem to be mostly connected to modifications in the GIT structure. Additionally, Kumar et al. (2020) showed that laminari-oligosaccharides have the potential to be used as prebiotic ingredients in functional food items. Furthermore, Ziółkowska et al. (2020) demonstrated that the results of blood parameters showed that the addition of prebiotics had no detrimental effects on the common carp's ability to grow or alter its homeostasis.

Prebiotics enhance Hb, Ht, and RBCs, leading to an increase in well-oxygenated blood reaching the tissue. Furthermore, fish with higher blood parameters, such as increased blood pressure, may exhibit stronger immune systems, indicated by higher WBC levels (Esmaeili 2021). These variables could explain the highly positive correlation observed between growth and the prebiotic substance utilized.

Liver enzymes serve as powerful indicators of the physiological condition of the liver, and monitoring enzyme activity is a biochemical approach to assess how food additives affect fish welfare and metabolism (Fadl et al. 2020). The present investigation revealed that the addition of prebiotic slightly decreased liver enzymes, reflecting the minimal burden of the applied prebiotic for *O. niloticus*.

Oxidative damage becomes more prevalent in stressful settings and infectious diseases that produce reactive oxygen species (ROS). This is attributed to differences in the production and disposal of free radicals (Martínez-Alvarez et al. 2005), and higher concentrations of ROS promote lipid peroxidation, as reflected in elevated concentrations of malondialdehyde (MDA) (Brewer 2011). The current investigation demonstrates a significant boost in oxidative enzymes, including CAT, GPx, and SOD, as well as notable reduction in MDA levels with the use of the prebiotic formula. This aligns with Wang et al. (2017), who showed that the addition of probiotics (*Bacillus licheniformis*) and 0.2% prebiotics (inulin) to water enhanced the development rate of juvenile groupers, along with increased activities of digestive enzymes and oxidative systems. Prebiotics can enhance fish's immune and antioxidant systems, significantly increasing the ability of aquaculture-raised animals to survive adverse environments (Jin et al. 2018). Additionally, Zhu et al. (2023) demonstrated that dietary mannan-oligosaccharide and xylooligosaccharide as prebiotics can greatly increase vitality against stress.

In a study of Zhang et al. (2021) that was conducted on mice, they found that an indigestible fructooligosaccharide (FOS) can prevent the impairment of the intestinal barrier caused by dextran sulfate sodium. Galacto-oligosaccharide (GOS), another indigestible oligosaccharide when consumed together with FOS, can increase the concentration of acetic acid, a short-chain fatty acid (SCFA), and the mRNA expression of G-coupled protein

receptors (GPR42 and GPR43), CLDN1, OCLN, and ZO1 in the colon of *neonatal piglets* (Zhang et al. 2020).

According to Uribe et al. (2011), LYZ, which is produced by leucocytes, is essential for the initiation of phagocytosis and has a bactericidal impact by lysis of the bacterial cell wall. Similarly, IgM plays a key role in the humoral immunological structure of fish (Tang et al. 2008). In this study, fish consuming prebiotics showed a substantial rise in lysozyme activity and IgM relative to the control. This is in line with Cavalcante et al. (2020), who demonstrated that probiotics, prebiotics, and synbiotics could be used to enhance growth performance and immune functions in Nile tilapia.

TNFα was initially discovered in fish as a single instance gene in the Japanese flounder's activated leukocytes. It is now recognized as a crucial regulatory cytokine in antibacterial defense and inflammatory responses (Grayfer et al. 2008). It is well-established that the fish *GH* and *IGF-I* genes play essential roles in controlling cell functions and growth through various signaling pathways. *IL-1β* functions as a chemoattractant for fish leucocytes, with leucocytes movement being regulated by G protein-coupled receptor activation and a progressive gradient of chemokines. Inflammatory areas attract and activate neutrophils through *IL-8* (Zou and Secombes 2016). In this study, the use of Fβ in various treatments resulted in the upregulation of *TNF* α, *IL-1 β*, *IL-8*, *GH*, and *IGF-1*, shifting the immune response toward a protective function. This may be one of the reasons for enhancing the tolerance of *O. niloticus* to *F. oxysporum* infection when using different levels of Fβ.

In this study, the histological investigations revealed improving the positive impacts of Fβ on the hepatopancreatic, intestinal, and splenic tissues of *O. niloticus* after the challenge test with *F. oxysporum*. This aligns with other studies reporting that the use of probiotics and prebiotics enhances the microscopic structures of digestive organs in fish (Ngamkala et al. 2020; Ruiz et al. 2020; Eissa et al. 2023c).

Conclusion

The study highlighted the efficacy of the fructooligosaccharides and β-1,3 glucan mixture addition, collectively referred to as Fβ, in improving fish health status. These improvements include enhanced growth performance, improved biochemical and hematological parameters, better organ health, and increased resistance against *F. oxysporum* infection. Moreover, it revealed a positive influence on the expression of developmental and defense genes, along with the histological structure of internal fish organs such as liver, intestine, and spleen. As a result, the inclusion of prebiotics into feed emerges as a secure and innovative approach for sustainable aquaculture, offering potential benefits for growth improvement, feed efficiency, and overall health in cultured Nile tilapia (*O. niloticus*). The author recommends incorporating Fβ at a level of 1–1.5g/kg diet to optimize fish health functions and growth.

Acknowledgements All authors extend their appreciation to their universities.

Author contributions Methodology, software: E.H.E., H.S.D.; conceptualization, visualization, methodology: E.H.E., N.M.N., F.A.J., O.F.A., and W.F.A.; software, validation, formal analysis: A.H.Q., F.A.J., O.F.A.; investigation, data curation: E.H.E., H.S.D.; histological processing, examination, and histological data interpretation: Y.M.S, H.S.D.; writing original draft preparation: Y.M.S, E.H.E.; writing review and editing: H.S.D., E.H.E.; supervision: A.R.; revised manuscript: E.H.E; project administration: E.H.E.; All authors have read and approved to the published version of the manuscript.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data availability All data regarding this study are presented in the paper.

Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of fish were followed by the authors and according to Suez University protocol (SUEZ Sci_IRB:21/04/2024/7)

Consent for publication All authors review and approve the manuscript for publication.

Competing interests The authors declare no competing interests.

Informed consent Not applicable.

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