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Original Article

Protective effect of *Ocimum gratissimum* leaf aqueous extract on antioxidant capacity, immunocompetence, and disease resistance of Nile tilapia (*Oreochromis niloticus*) fingerlings against Vibrio parahaemolyticus infection

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Abstract: Infectious diseases are major constraints on the aquaculture industry, and they frequently arise from stress factors associated with intensive aquaculture practices. This study was carried out to assess the protective effect of *Ocimum gratissimum* leaf aqueous extract on immunological responses, antioxidant capacity, and disease resistance of *Oreochromis niloticus* fingerlings against *Vibrio parahaemolyticus* infection. Five iso-nitrogenous (30.02%) diets containing 0 (control), 2, 4g, 8, and 16 g *Ocimum*-leaf extract (g/kg) were fed to triplicate groups of fish (initial weight 3.31±0.05g) for 90 days. After a 90-day feeding trial, test fish were injected with pathogenic *V. parahaemolyticus*, and survival was recorded over the next 14 days. The white blood cell (12.29±0.35×10^3/µl), red blood cells (3.60±0.21×10^3/µl), packed cell volume (27.76±0.08 g/dl), and haemoglobin (7.21±0.47 g/dl) were all highest in fish fed 8 g/kg of *Ocimum* leaf extract. The biochemical parameters, such as alkaline phosphatase (ALP), total protein, albumin, and globulin contents, increased significantly in *Ocimum* leaf extracts fed groups compared with the control; these groups also registered the lowest values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA). Following a challenge with *V. parahaemolyticus,* the study indicated that *Ocimum* leaf extract reduced metabolic stress in infected fish compared with the control. There is a significant increase in glucose (101.00±2.89 mg/dl) levels and liver enzymes ALT (74.14±2.31 UI/ L) and AST (49.00±1.73 UI/L) in the control group (0 g/kg), whereas the opposite was true for diets supplemented with *Ocimum* leaf extract. The *Ocimum* leaf extract group significantly enhanced immune parameters, including lysozyme, immunoglobulin (IgM), and Interleukin-1β (IL-1β), in the spleen, skin, and kidney. After infection, the supplemented diets containing Ocimum leaf extract showed significantly higher lysozyme and IgM activities in the spleen, skin, and kidney. The result of the challenge test with *V. parahaemolyticus* revealed that the highest survival (80%) was recorded in the group fed *Ocimum*-supplemented diets at 8 and 16 g/kg, and the lowest skin lesion morbidity (15%) was observed in fish fed 8 g/kg. This study revealed that the inclusion of *O. gratissimum* leaf extract in the diet of *O. niloticus* enhanced biochemical parameters and immune response, and improved resistance to *V. parahaemolyticus*.

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Introduction

Aquaculture is currently one of the fastest-growing food-producing sectors worldwide and plays a pivotal role in global food security, human nutrition, and socioeconomic development (Abeysinghe et al., 2025). The sector provides high-quality animal protein while also generating employment opportunities and income for millions of people, particularly in developing countries (Liboureau et al., 2026; Nordhagen et al., 2026). Among cultured

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aquatic species, Nile tilapia (*Oreochromis niloticus*) is recognized as one of the most economically important freshwater fish species worldwide, owing to its rapid growth rate, high consumer acceptance, adaptability to diverse environmental conditions, and efficient utilization of relatively inexpensive feeds (El-Sayed and Fitzsimmons, 2023; Vilhena Batista et al., 2026). Consequently, Nile tilapia contributes substantially to global aquaculture production and serves as an affordable and accessible source of animal protein in many tropical and subtropical regions (Koley et al., 2025). However, as with other intensively farmed fish species, the continuous expansion and intensification of tilapia farming have significantly increased fish vulnerability to infectious diseases, particularly under stressful rearing conditions and inadequate biosecurity practices (Chupani et al., 2026; Emam et al., 2025).

Disease outbreaks in tilapia culture systems frequently result in physiological stress, suppressed growth performance, reduced survival, and considerable economic losses for producers, while disease infestation and growth retardation remain among the principal factors limiting tilapia productivity in Nigeria and several other developing countries (Paredes-Trujillo and Mendoza-Carranza, 2022; Maezono et al., 2025; Ndaro et al., 2026). Among the bacterial diseases affecting cultured fish, vibriosis caused by *Vibrio* species is regarded as one of the most devastating infectious diseases in aquaculture worldwide (Sanches-Fernandes et al., 2022; Manchanayake et al., 2023; Camberos-Solano and Soto-Rodriguez, 2026). *Vibrio parahaemolyticus*, a Gram-negative opportunistic pathogen, can infect marine, brackish, and freshwater fish species, leading to septicemia, skin hemorrhage, tissue necrosis, and high mortality rates (Petrusic et al., 2025; Li et al., 2026). The disease is particularly severe in fry and fingerlings due to their relatively immature immune systems and lower resistance to pathogenic infections. Furthermore, *V. parahaemolyticus* is recognized as an important foodborne pathogen that poses substantial risks to both aquaculture production and public health (Zaher et al., 2021; ELTarabili et al., 2026). Therefore, improving the immune competence and disease resistance of cultured fish through nutritional interventions and functional feed-based strategies has become an important, sustainable approach to enhancing fish health and supporting the long-term sustainability of aquaculture production systems.

In recent years, plant-derived feed additives and phytogenic compounds have attracted considerable attention in aquaculture as promising alternatives to synthetic antibiotics and chemotherapeutic agents (Ivanova et al., 2024; Bai et al., 2025; Pudota et al., 2025). Medicinal plants and their derivatives are rich sources of biologically active compounds, including flavonoids, phenolics, alkaloids, terpenoids, tannins, and essential oils, which exhibit antimicrobial, antioxidant, anti-inflammatory, and immune-stimulatory properties (Chakroborty et al., 2025; Hossain et al., 2026). Owing to these functional characteristics, dietary supplementation with phytogenic additives has been widely reported to enhance growth performance, feed utilization efficiency, antioxidant defense systems, immune responses, gut health, and disease resistance in cultured fish species (Hu et al., 2025; Ibrahim et al., 2025; Adhikary et al., 2026; Soares et al., 2026; Xi et al., 2026). Moreover, phytogenic feed additives are generally biodegradable, environmentally friendly, and safe for long-term use, making them attractive candidates for sustainable, antibiotic-free aquaculture production systems (Wang et al., 2024; Hassan et al., 2025).

Among the medicinal plants investigated for aquaculture applications, *Ocimum gratissimum* L. has emerged as a particularly promising phytogenic additive because of its broad spectrum of biological activities (Bandeira et al., 2017). *Ocimum gratissimum*, commonly known as clove basil or scent leaf, is an aromatic medicinal plant widely distributed throughout tropical and subtropical regions, especially in Africa and Asia (Ugbogu et al., 2021). The plant has long been used in traditional medicine for its diverse pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and immune-modulatory effects (Kamelnia et al., 2023; Sagar et al., 2025). Previous studies have demonstrated that

O. gratissimum contains several important bioactive compounds, such as eugenol, thymol, linalool, methyl cinnamate, camphor, flavonoids, and terpenoids, which are largely responsible for its biological activities (Leite dos Santos et al., 2025; Odeniyi et al., 2025; Akanbi et al., 2026; Sharma et al., 2026). Additionally, Egba et al. (2014) reported that extracts of *O. gratissimum* enhanced both primary and secondary antibody responses and stimulated immune functions. In aquaculture, dietary administration of *O. gratissimum* has been associated with improvements in hematological parameters, antioxidant capacity, immune responses, and disease resistance (Abdel-Tawwab et al., 2018; Ballester et al., 2025). Collectively, these findings suggest that *O. gratissimum* could serve as an effective natural immunostimulant and antioxidant feed additive capable of enhancing fish health and resistance against pathogenic infections. However, despite the increasing interest in phytogenic feed additives, information regarding the protective effects of *O. gratissimum* leaf aqueous extract against *V. parahaemolyticus* infection in Nile tilapia remains limited, particularly concerning its influence on hematological responses, oxidative stress biomarkers, innate immune parameters, and post-challenge disease resistance. Therefore, the present study was conducted to evaluate the protective effects of dietary *O. gratissimum* leaf aqueous extract on hematological responses, serum biochemical parameters, antioxidant status, innate immune responses, and disease resistance of Nile tilapia fingerlings challenged with *V. parahaemolyticus*.

Materials and Methods

Collection of leaf samples: Fresh leaves of *O. gratissimum* were collected from Saye Village, located in Zaria Local Government Area, Kaduna State, Nigeria. The plant material was taxonomically authenticated at the Herbarium Unit, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, where a voucher specimen was deposited under the accession number ABU01285.

Preparation of aqueous extract of *O. gratissimum*: The collected leaves of *O. gratissimum* were dried and ground into a fine powder. Briefly, 2500 g of the powdered sample was macerated in 2.5 L of distilled water at room temperature for 24 h. The resulting mixture was subsequently filtered through Whatman No. 1 filter paper to remove particulate materials. The filtrate was then concentrated in a water bath maintained at 40°C until a semi-solid extract was obtained. The prepared aqueous extract was transferred into sterile sample bottles and stored under appropriate conditions until further use, following the method described by Salau et al. (2013).

Determination of phytochemical components of *O. gratissimum* leaf aqueous extract: The phytochemical composition of *O. gratissimum* leaf aqueous extract was determined using Gas Chromatography–Mass Spectrometry (GC-MS) analysis conducted at the Multiuser Science Laboratory, Department of Chemistry, Ahmadu Bello University. The analysis was performed to identify and characterize the bioactive compounds present in the aqueous extract (Table 1), while the corresponding total ion chromatogram is presented in Figure 1.

GC-MS analysis was carried out using a 7890B GC System coupled with a 5977A Mass Selective Detector (Agilent Technologies, USA). Prior to analysis, the semi-solid extract of *O. gratissimum* was diluted in methanol, and 2 μL of the prepared solution was injected into the GC-MS system using a microsyringe. The operating conditions of the GC-MS system were as follows: initial oven temperature of 70°C with an equilibration time of 1 min, followed by a programmed increase at 5°C min^{-1} to 250°C, then maintained for 1 min, and finally increased at 30°C min^{-1} to 300°C. The maximum oven temperature was set at 325°C, with a post-run temperature of 50°C. Helium was used as the carrier gas under split injection mode. The injector temperature was maintained at 250°C with a pressure of 11.089 psi, a total flow rate of 19.204 mL min^{-1}, a septum purge flow of 3 mL min^{-1}, and a purge flow to the split vent of 15 mL min^{-1} at 0.75 min. The hold-up time and carrier gas flow rate were 1.2386 min and 1.0 mL

Table 1. GC-MS profile of *Ocimum gratissimum* leaf aqueous extract.

Retention Time (minute)	% Peak Area	Name of Compounds	Reference
5.80	0.17	Eucalyptol	27465
6.03	0.06	Oxirane, tetradecyl-	102576
6.39	0.21	Benzaldehyde, 2-hydroxy-	10083
8.00	0.07	Hexanoic acid, methyl ester	13783
10.89	0.29	Methenamine	18654
12.26	0.28	3-Hexyne-2,5-diol, 2,5-dimethyl-	20272
12.89	0.85	Thymol	24339
17.75	0.36	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	68686
18.53	0.30	Methyl 8-methyl-decanoate	65001
20.17	0.15	Fluoroacetic acid, dodecyl ester	107808
20.35	0.07	2-Methyltetracosane	207500
23.37	0.22	Tridecanoic acid, methyl ester	91455
24.88	0.30	Heptadecyl trifluoroacetate	207045
25.02	0.11	Hexadecane, 1-chloro-	121205
25.47	0.19	Phenol, 2,6-diamino-	10528
25.70	0.23	3-Pentanone, 2,4-dimethyl-, O-methyloxime	20882
26.71	0.13	Oxirane, decyl-	51291
27.81	2.88	Pentadecanoic acid, 14-methyl-, methyl ester	130841
28.69	0.29	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	138186
29.17	0.23	Pentafluoropropionic acid, tetradecyl ester	213156
31.06	0.98	1-Hexadecanol	104431
31.24	1.25	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	153891
31.36	5.43	9-Octadecenoic acid (Z)-, methyl ester	155751
31.64	0.55	Phytol	155851
31.85	2.76	Methyl stearate	157880
32.64	0.09	7,11-Hexadecadienal	98680
32.81	0.28	1-Isopropoxy-2,2, 3-trimethylaziridine (anti)	20887
33.08	0.36	17-Pentatriacontene	265113
33.58	0.06	Pentanoic acid, 10-undecenyl ester	115375
34.02	0.07	2-Methyl-Z,Z-3,13-octadecadienol	140253
35.56	0.34	Undecanoic acid, 2-methyl-	65005
35.68	0.16	9,12-Octadecadienoyl chloride, (Z,Z)-	157778
35.81	0.17	Oleic Acid	142069
36.13	0.44	2-Methyl-Z,Z-3,13-octadecadienol	140253
36.71	0.10	Cyclohexanemethanol	7618
36.83	0.62	Hexanedioic acid, bis(2-ethylhexyl) ester	220809
37.81	60.57	9,17-Octadecadienal, (Z)-	125003
38.36	18.41	Benzoic acid, 4-amino-2-propoxy-, 2-(diethylamino)ethyl ester	153490

min^{-1}, respectively.

Experimental diet preparation: The feed ingredients used in diet formulation were purchased from Sabon-Gari Market in Zaria, Kaduna State, Nigeria. All ingredients were cleaned, processed, and finely milled prior to diet preparation. Five experimental diets (Diets 1-5) were formulated to be isonitrogenous, containing 29.78-30.39% crude protein (Table 2), and supplemented with different inclusion levels of *O. gratissimum* leaf aqueous extract at 0, 2, 4, 8, and 16 g kg^{-1} diet.

The basal diet (0 g kg^{-1} *O. gratissimum* extract; control diet) was prepared using yellow maize, wheat bran, soybean meal, fish meal, corn starch, chicken intestine, palm oil, vitamin–mineral premix, lysine, and methionine. The ingredients were thoroughly mixed with water to form a homogeneous dough, then pelletized, air-dried, and subsequently crushed into pellets of appropriate size for the experimental fish. The supplemented diets were prepared using the same basal formulation with the respective inclusion levels of *O. gratissimum* leaf aqueous extract.

During the feeding trial, fish were fed the experimental diets to apparent satiation three times

Table 2. Composition of the experimental diets with levels of *Ocimum gratissimum* leaf extract.

	Ocimum leaf extract levels (g/kg diet)				
Ingredients (kg)	0	2	4	8	16
Yellow corn	350	350	350	350	350
Soya bean meal	200	200	200	200	200
Wheat bran	60	60	60	60	60
Corn starch	100	100	100	100	100
Fish meal	75.0	75.0	75.0	75.0	75.0
Chicken intestine	150	150	150	150	150
Palm oil	30	30	30	30	30
Premix[a]	15.0	15.0	15.0	15.0	15.0
Lysine	10	10	10	10	10
Methionine	10	10	10	10	10
Proximate composition (%)					
Crude protein	29.78	30.07	29.84	30.00	30.39
Crude lipid	8.23	8.12	8.50	8.64	8.23
Nitrogen free extract	34.78	33.22	33.71	34.02	34.56
Crude fibre	11.06	12.06	12.00	12.00	12.67
Ash	6.99	7.01	6.98	7,79	7.18

Premix[a]: vitamin and mineral premix (IU or mg/kg). Vitamin A, 4800 IU; vitamin D3, 2400 IU; vitamin E, 4000 mg; vitamin K: 800 mg; Vitamin B1: 400mg; vitamin B2, 1600 mg; vitamin B6, 600 mg; vitamin B12, 4 mg; pantothenic acid, 4000 mg; nicotinic acid, 8000mg; folic acid, 400 mg; biotin, 20 mg, Manganese, 22000 mg; zinc, 22000 mg; iron, 12000 mg; copper, 4000 mg; iodine, 400 mg; selenium, 400mg; cobalt, 4.8 mg.

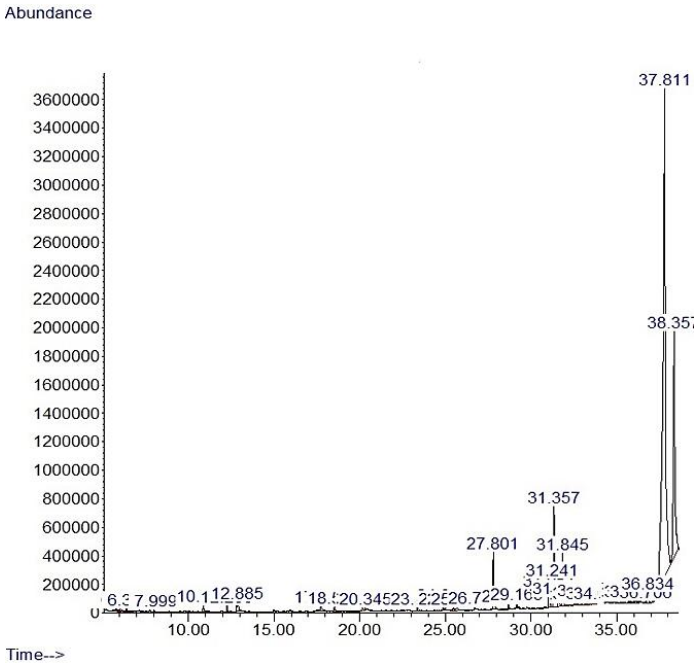

Figure 1. Total ion chromatogram of *Ocimum gratissimum* leaf aqueous extract

daily for 90 days. The experimental diets were weighed before the commencement and at the end of the feeding trial to estimate feed intake and determine the amount of feed administered to each experimental tank.

Experimental fish and rearing conditions: A total of 800 healthy fingerlings of Nile tilapia were obtained from a commercial fish farm in Kano State, Nigeria. The fish were transported in 50-L plastic containers to the Fisheries Research Laboratory, Department of Biology, Faculty of Life Sciences, Ahmadu Bello University. Upon arrival, the fish were acclimatized in holding tanks containing dechlorinated tap water and fed a commercial diet (Coppens) at 5% of body weight for two weeks prior to the commencement of the feeding trial.

After acclimation, fish with an initial mean body weight ranging from 3.28±0.03 to 3.36±0.05 g were randomly distributed into plastic experimental tanks (30.0×20.0×25.0 cm) containing 35 L of water. Each tank was stocked with 15 fish, and each dietary treatment was conducted in triplicate, resulting in 45 fish per treatment group. The fish were assigned to one of five experimental diets containing 0 (control), 2, 4, 8, or 16 g kg^{-1} of *O. gratissimum* leaf aqueous extract.

Before the initiation of the feeding trial, all fish were batch-weighed and measured to determine their initial body weight and total length using a top-loading digital balance (AT. M A 123). Final measurements were similarly recorded at the end of the 90-day experimental period.

Determination of hematological parameters: At the

end of the 90-day feeding trial, three fish were randomly sampled from each experimental tank for hematological analysis. Prior to sampling, fish were fasted for 24 h to minimize postprandial variations in blood biochemical and hematological parameters. Blood samples were collected from the heart and caudal peduncle using 1-mL hypodermic syringes pre-rinsed with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant.

For serum collection, nine fish per treatment were euthanized using buffered benzocaine solution (>250 mg L^{-1}). Fish were maintained in the anesthetic solution for approximately 10 min following cessation of opercular movement to ensure complete euthanasia. Hematological analyses were subsequently performed using a Biobase BK6100 Hematology Analyzer (Biobase, China) operated in whole-blood and predilute modes.

The hematological parameters evaluated included white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), and packed cell volume (PCV). In addition, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the methods described by Dacie and Lewis (1975) using the formula of MCV (μm^3) = Ht / (RBC's) ×10, and MCH (pg) = Hb / (RBC's) × 10.

Serum biochemical assessment for liver enzymes: Serum biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, glucose, and globulin, were determined using commercial colorimetric assay kits (Randox Laboratories Ltd., London, UK) following the manufacturer's instructions. The absorbance values of the standards and samples were measured relative to a reagent blank using a spectrophotometer at 630 nm.

Sample preparation for oxidative biomarkers and immune parameters: Skin mucus samples were collected by gently scraping the dorsal body surface of the experimental fish using sterile glass slides. Following mucus collection, the fish were dissected along the left lateral side to expose the abdominal cavity, and tissue samples, including liver, spleen, and kidney, were carefully excised. The collected tissue samples and skin mucus were each homogenized in physiological saline solution and then centrifuged at 6000 × g for 20 min at 4°C. The resulting supernatants were carefully collected and stored under appropriate conditions for subsequent analyses of oxidative stress biomarkers and immune-related parameters.

Determination of oxidative stress biomarkers: Liver tissues of *O. niloticus* were collected and immediately perfused with chilled physiological saline solution (0.9% NaCl). The tissues were homogenized in physiological saline, filtered, and centrifuged at 1500 rpm for 20 min at 4°C. The resulting clear supernatants were collected and used for analyses of oxidative stress biomarkers. Total protein content in the supernatant was determined and expressed relative to the wet tissue weight. The oxidative stress biomarkers analyzed included reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA), following the methods described by Nishikimi et al. (1972), Beutler et al. (1963), Aebi (1984), and Ohkawa et al. (1979), respectively. Commercial assay reagents and kits were obtained from Biodiagnostic Co.

Reduced glutathione (GSH) levels were determined spectrophotometrically using a Photometer 5010 (BM Co., Germany) according to the method of Beutler et al. (1963). Catalase (CAT) activity was assessed by measuring the rate of hydrogen peroxide decomposition at 240 nm, following the procedure described by Aebi (1984). Superoxide dismutase (SOD) activity was determined by diluting 0.1 mL of tissue homogenate with 0.9 mL distilled water to obtain a 1:10 dilution. Subsequently, 0.2 mL of the diluted sample was mixed with 2.5 mL of 0.05 M carbonate buffer, followed by the addition of 0.3 mL of 0.3 mM adrenaline solution. The reference mixture consisted of 2.5 mL of carbonate buffer, 0.3 mL of adrenaline solution, and 0.2 mL of distilled water. Absorbance was measured at 480 nm from 30 to 150 s, and SOD activity was calculated based on the percentage inhibition of adrenaline auto-

oxidation.

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) as described by Ohkawa et al. (1979). Briefly, 2 mL of 15% trichloroacetic acid and 2 mL of thiobarbituric acid solution were added to 100 µL of tissue homogenate. The mixture was incubated in a water bath at 80°C for 30 min, cooled to room temperature, and centrifuged at 3000 rpm for 10 min. The absorbance of the resulting supernatant was measured at 535 nm using a spectrophotometer. The concentration of TBARS was expressed as nmol/mg protein.

Determination of immune parameters: Immune-related parameters were assessed in the spleen, skin, and kidney tissues of the experimental fish using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. The evaluated immune parameters included lysozyme activity (Fish LZM CK-LAB Kit), immunoglobulin M (IgM; Fish IgM CK-LAB Kit), and interleukin-1β (IL-1β; Interleukin 1 Beta FineTest ELISA Kit). The lysozyme and IgM assay kits were obtained from Shanghai Coon Koon Biotech Co., Ltd. All assays were performed according to the manufacturers' protocols.

Challenge test of experimental fish with *V. parahaemolyticus*: At the end of the 90-day feeding trial, the experimental fish were challenged with a virulent strain of *V. parahaemolyticus*. The bacterial strain was obtained from the Department of Veterinary Microbiology, Ahmadu Bello University. The bacterium was cultured on blood agar plates and incubated at 37°C for 24 h prior to the challenge test. A preliminary experiment was conducted to determine the median lethal dose (LD_{50}) of *V. parahaemolyticus*. Fish were intraperitoneally injected with 0.1 mL of bacterial suspensions containing different concentrations of *V. parahaemolyticus* (1.5×10^8, 3.5×10^8, 6×10^8, and 9×10^8 CFU mL^{-1}). Bacterial density was quantified spectrophotometrically at an optical density (OD) of 450 nm. The results of the preliminary trial indicated that 0.1 mL containing 3.7×10^8 CFU mL^{-1} represented the 96-h LD_{50} dose.

For the challenge experiment, 10 healthy fish were randomly selected from each treatment tank and intraperitoneally injected with 0.1 mL phosphate-buffered saline (PBS) containing 3.7×10^8 CFU mL^{-1} of *V. parahaemolyticus*. Prior to bacterial challenge, fish were fasted for 24 h, after which feeding with the respective experimental diets was resumed. Following infection, fish were monitored daily for 14 days to record mortality and observe abnormal clinical signs. At the end of the challenge period, the severity of skin hemorrhage and lesions was evaluated using a point-based grading system as described by Liu et al. (2018).

Water quality monitoring: Water quality parameters were monitored throughout the experimental period to ensure suitable rearing conditions for the fish. Water temperature (27.31-27.48°C), pH (7.29-7.37), electrical conductivity (206.67 µS cm^{-1}), and total dissolved solids (103.33-105.33 ± 1.26 mg L^{-1}) were measured daily using a Hanna multiparameter instrument (Model No. 626 973-01). Dissolved oxygen concentration (7.13±0.17 mg L^{-1}) was determined according to the standard methods described by APHA (2007).

Data analysis: All data were initially tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. Subsequently, the collected data were analyzed using one-way analysis of variance (ANOVA) in R (version 4.1.2). When significant differences were detected, Tukey's multiple comparison test was applied to compare treatment means. Differences were considered statistically significant at $P<0.05$. In addition, dependent t-tests were performed to compare parameters before and after the bacterial challenge. Graphs were generated using GraphPad Prism software (version 9.0).

Results

Hematological parameters: The hematological parameters of *O. niloticus* fed diets supplemented with different levels of *O. gratissimum* leaf extract before and after challenge with *V. parahaemolyticus* are presented in Figure 2. White blood cell (WBC) counts were significantly higher ($P<0.05$) in all dietary

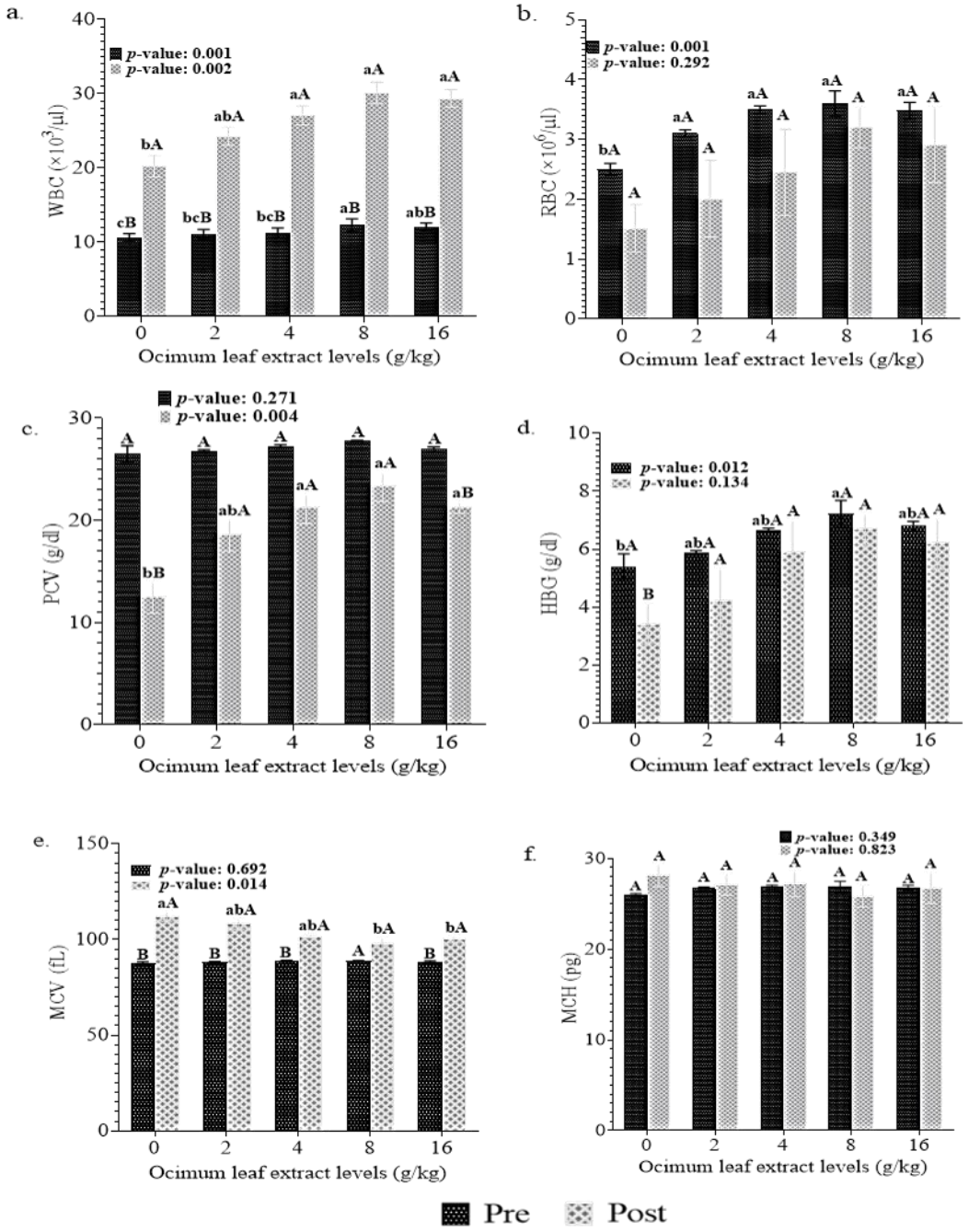

Figure 2. Haematological parameters. (a) White blood cell, (b) Red blood cell, (c) Packed cell volume, (d) Haemoglobin, (e) Mean corpuscular volume, and (f) Mean corpuscular haemoglobin) of *Oreochromis niloticus* fed a diet with different levels of *Ocimum gratissimum* leaf extract pre- and post-challenged with *Vibrio parahaemolyticus*. Values are mean±SE, bars with different letters (a, b, c) differ significantly at $P< 0.05$. Upper case letters (A and B) indicated a significant difference at $P<0.05$ between pre- and post-challenging with *V. parahaemolyticus*

treatment groups compared with the control group under both pre- and post-challenge conditions, with the highest values observed in fish fed 8 g kg^{-1} *Ocimum* leaf extract. Furthermore, WBC levels increased significantly following the bacterial challenge, indicating an enhanced immune response against *V. parahaemolyticus*. Similarly, red blood cell (RBC) counts were significantly elevated ($P<0.05$) in fish fed *Ocimum*-supplemented diets compared with the control group prior to challenge. However, no significant differences ($P>0.05$) in RBC values were observed among the dietary groups following bacterial infection.

Hemoglobin (HGB) was also significantly higher

(*P<0.05*) in fish receiving *Ocimum* leaf extract-supplemented diets than in the control group during the pre-challenge period. The highest HGB value (7.21±0.47 g dL^{-1}) was recorded in fish fed 8 g kg^{-1} *Ocimum* leaf extract, which was comparable to the values observed in fish fed 16 g kg^{-1} (6.79±0.17 g dL^{-1}), 4 g kg^{-1} (6.65±0.08 g dL^{-1}), and 2 g kg^{-1} (5.89±0.06 g dL^{-1}), but significantly higher than that of the control group (5.37±0.47 g dL^{-1}). Following challenge with *V. parahaemolyticus*, a marked reduction in HGB concentration was observed in the control group, decreasing from 5.37±0.47 to 3.43±0.69 g dL^{-1}, whereas no significant differences ($P>0.05$) were detected among the treatment groups. Overall, WBC, RBC, PCV, and HGB values increased progressively with increasing dietary inclusion levels of *Ocimum* leaf extract up to 8 g kg^{-1}, after which a declining trend was observed at 16 g kg^{-1} under both pre- and post-challenge conditions. However, no significant differences ($P>0.05$) were observed in PCV, (MCV), or MCH between the experimental groups and the control group.

Serum biochemical assessment for liver enzymes: The serum biochemical parameters of *O. niloticus* fed diets supplemented with different levels of *O. gratissimum* leaf extract before and after challenge with *V. parahaemolyticus* are presented in Figure 3. ALT, AST, and glucose levels were significantly higher ($P<0.05$) in the control group compared with fish fed *Ocimum*-supplemented diets under both pre- and post-challenge conditions. In addition, the control group exhibited a significant increase ($P<0.05$) in ALT and glucose levels following challenge with *V. parahaemolyticus*, indicating increased physiological stress and possible hepatic damage. In contrast, fish receiving diets supplemented with *Ocimum* leaf extract maintained lower ALT, AST, and glucose values throughout the experimental period.

ALP activity and albumin concentration were significantly lower ($P<0.05$) in the control group compared with fish fed *Ocimum*-supplemented diets. Total protein levels were significantly enhanced in the supplemented groups, with the highest values recorded in fish fed 16 g kg^{-1} *Ocimum* leaf extract during the pre-challenge period (3.93 ± 0.52 g dL^{-1}) and in fish fed 8 g kg^{-1} during the post-challenge period (4.57±0.26 g dL^{-1}). In contrast, the lowest total protein values were observed in the control group, with 1.24±0.10 g dL^{-1} and 0.70±0.35 g dL^{-1} recorded under pre- and post-challenge conditions, respectively.

Similarly, globulin concentrations were significantly higher ($P<0.05$) in fish fed *Ocimum*-supplemented diets compared with the control group. The highest globulin values were recorded in fish fed 8 g kg^{-1} *Ocimum* leaf extract, reaching 2.87±0.09 g dL^{-1} and 2.99±0.43 g dL^{-1} under pre- and post-challenge conditions, respectively, whereas the control group exhibited the lowest values (0.79±0.10 and 0.77±0.30 g dL^{-1}, respectively). Albumin concentrations followed a similar trend, with the highest values observed in fish fed 8 g kg^{-1} *Ocimum* leaf extract during the pre-challenge period (1.98±0.16 g dL^{-1}) and 4 g kg^{-1} during the post-challenge period (1.70±0.29 g dL^{-1}). Conversely, the control group consistently recorded the lowest albumin values, with 0.55±0.17 and 0.35±0.14 g dL^{-1} under pre- and post-challenge conditions, respectively.

Oxidative stress biomarkers: The oxidative stress biomarkers in the liver of *O. niloticus* fed diets supplemented with different levels of *O. gratissimum* leaf extract before and after challenge with *V. parahaemolyticus* are presented in Figure 4. SOD activity was significantly higher ($P<0.05$) in fish fed *Ocimum*-supplemented diets compared with the control group under both pre- and post-challenge conditions. The highest SOD activities were recorded in fish fed 8 g kg^{-1} *Ocimum* leaf extract, with values of 54.92±2.59 IU mL^{-1} and 55.27±2.29 IU mL^{-1} before and after challenge, respectively, whereas the control group exhibited the lowest values (33.30±2.03 and 35.15±1.29 IU mL^{-1}, respectively).

A similar pattern was observed for CAT activity, which increased significantly in fish receiving *Ocimum*-supplemented diets. The highest CAT activities were observed in fish fed 16 g kg^{-1} (17.46±1.25 U mL^{-1}) and 8 g kg^{-1} (15.90±2.08 U

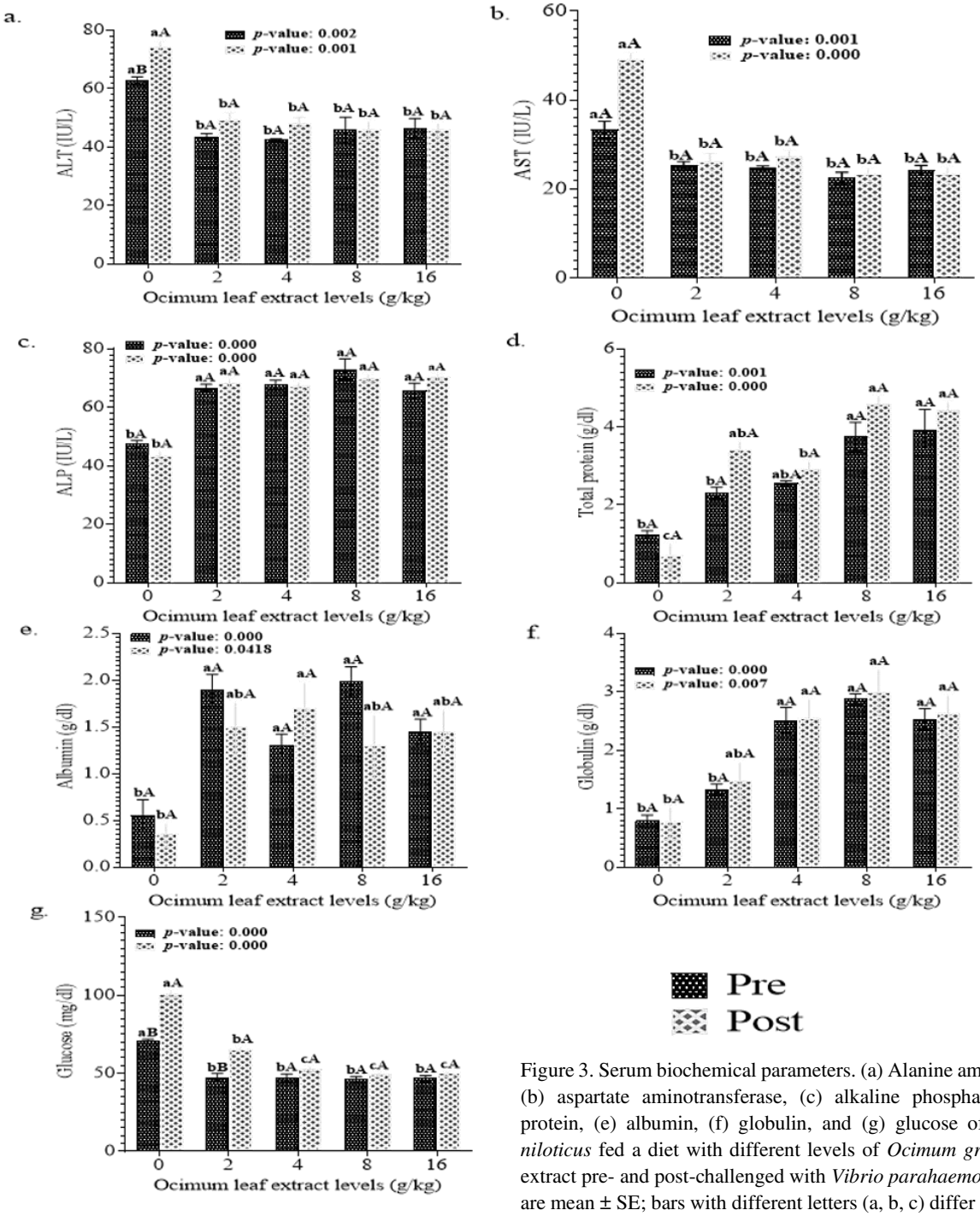

Figure 3. Serum biochemical parameters. (a) Alanine aminotransferase, (b) aspartate aminotransferase, (c) alkaline phosphatase, (d) total protein, (e) albumin, (f) globulin, and (g) glucose of *Oreochromis niloticus* fed a diet with different levels of *Ocimum gratissimum* leaf extract pre- and post-challenged with *Vibrio parahaemolyticus*. Values are mean ± SE; bars with different letters (a, b, c) differ significantly at $P<0.05$. Upper case letters (A, B) indicated a significant difference at $P<0.05$ between pre- and post-challenging with *V. parahaemolyticus*.

mL^{-1}) *Ocimum* leaf extract during the pre- and post-challenge conditions, respectively. In contrast, malondialdehyde (MDA) levels, an indicator of lipid peroxidation and oxidative damage, were significantly reduced ($P<0.05$) in fish fed *Ocimum*-supplemented diets compared with the control group. The lowest MDA concentrations were recorded in fish fed 16 g kg^{-1} (27.33±0.73 nmol mL^{-1}) and 8 g kg^{-1} (38.80±2.52 nmol mL^{-1}) *Ocimum* leaf extract under pre- and post-challenge conditions, respectively.

Reduced glutathione (GSH) levels were also significantly elevated ($P<0.05$) in fish fed *Ocimum* leaf extract, with the highest values observed in the 8 and 16 g kg^{-1} dietary groups under both pre- and post-

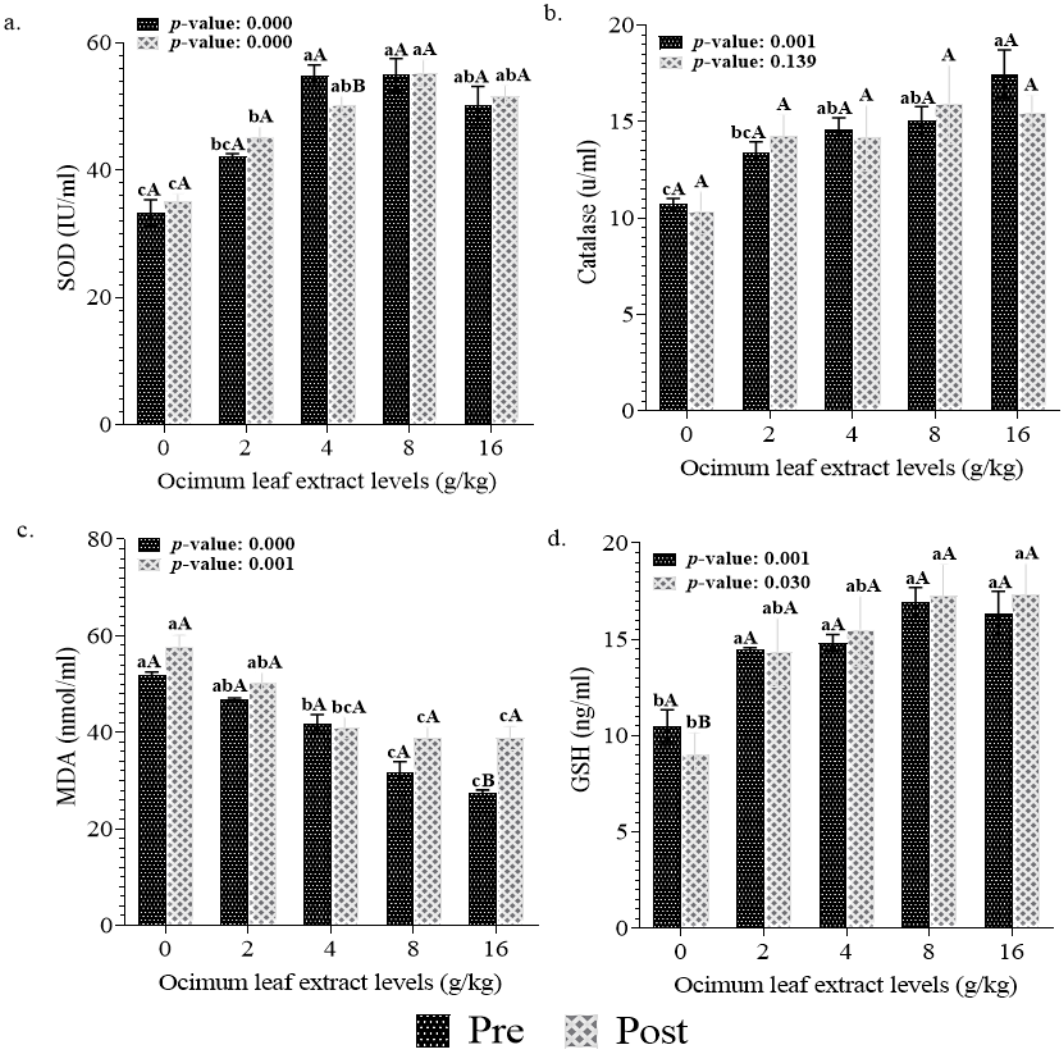

Figure 4. Oxidative stress biomarkers in the liver. (a) Superoxide dismutase, (b) catalase, (c) malondialdehyde, and (d) reduced glutathione of *Oreochromis niloticus* fed a diet with different levels of *Ocimum gratissimum* leaf extract pre- and post-challenged with *Vibrio parahaemolyticus*. Values are mean ± SE; bars with different letters (a, b, and c) differ significantly at $P<0.05$. Uppercase letters (A and B) indicated a significant difference at $P<0.05$ between pre-and post-challenged with *V. parahaemolyticus*.

challenge conditions. Conversely, a significant reduction ($P<0.05$) in GSH levels was observed in the control group following *V. parahaemolyticus* challenge, indicating increased oxidative stress in untreated fish.

Immune parameters: Significant differences ($P<0.05$) in lysozyme activity were observed in the spleen, skin, and kidney tissues of *O. niloticus* fed diets supplemented with different levels of *O. gratissimum* leaf extract before and after challenge with *V. parahaemolyticus* (Fig. 5). Among the examined tissues, lysozyme activity was highest in the kidney, followed by the spleen, and lowest in the skin. In all three organs, lysozyme activity increased progressively with increasing dietary inclusion levels of *Ocimum* leaf extract. Following bacterial challenge, the highest lysozyme activity was observed in fish fed 8 g kg^{-1} *Ocimum* leaf extract in the kidney, whereas peak values in the spleen and skin were recorded in fish fed 16 g kg^{-1}. Overall, fish fed *Ocimum*-supplemented diets exhibited significantly higher ($P<0.05$) lysozyme activity in all examined tissues compared with the control group under both pre- and post-challenge conditions. However, no significant differences ($P>0.05$) in lysozyme activity were detected between pre- and post-challenge conditions within the same dietary groups, irrespective of tissue type.

The immunoglobulin M (IgM) levels in the spleen, skin, and kidney are presented in Figure 6. Fish fed

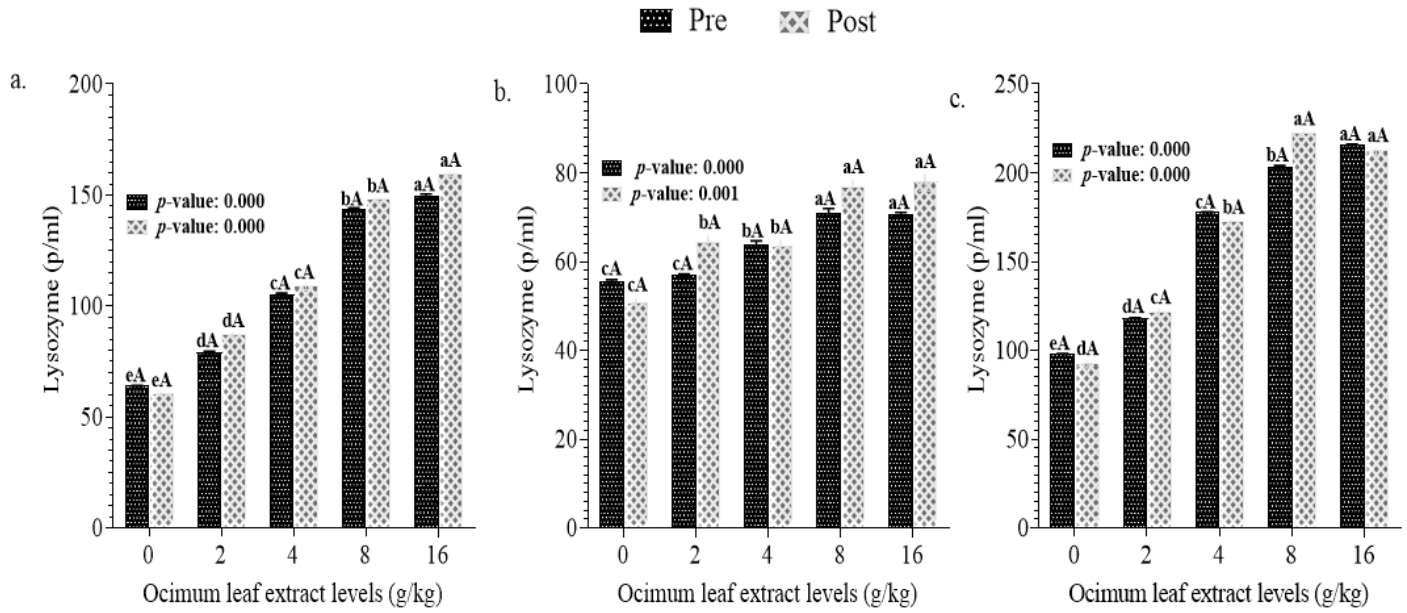

Figure 5. Lysozyme activity of (a) the spleen, (b) skin, and (c) kidney of *Oreochromis niloticus* fed a diet with different levels of *Ocimum gratissimum* leaf extract pre- and post-challenge with *Vibrio parahaemolyticus*. Values are the mean ± SE; bars with different letters (a, b, and c) differ significantly at $P<0.0$. Uppercase letters (A and B) indicated a significant difference at $P<0.05$ between pre- and post-challenge with *V. parahaemolyticus*.

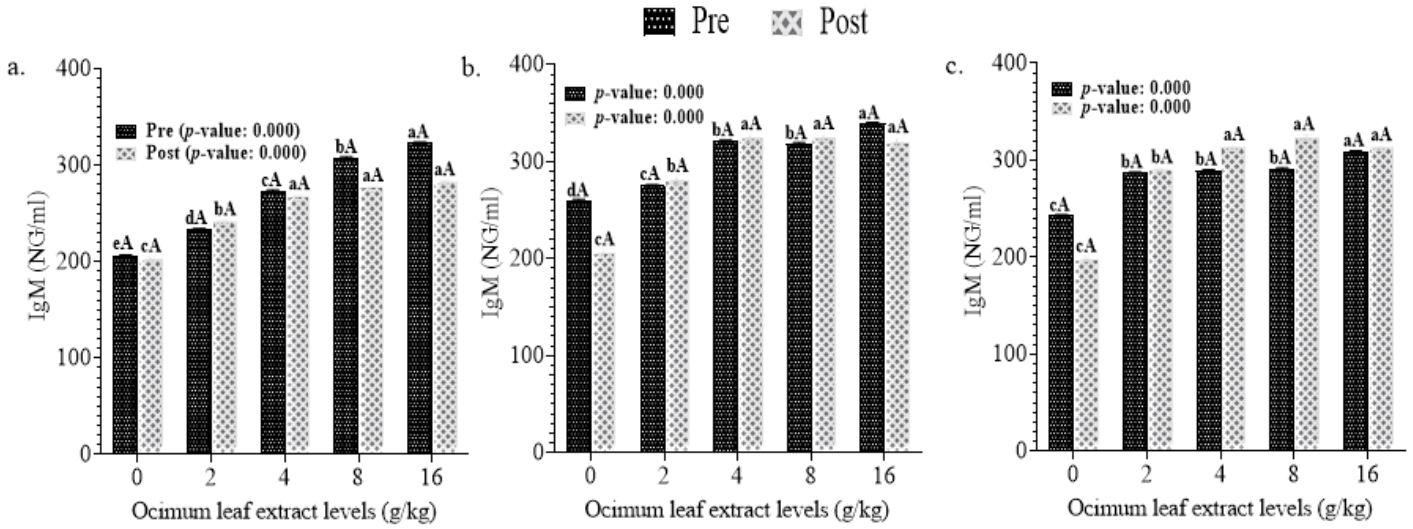

Figure 6. Immunoglobulin (IgM) activity (a) in the spleen, (b) in the skin, and (c) in the kidney of *Oreochromis niloticus* fed a diet with different levels of *Ocimum gratissimum* Leaf Extract pre- and post-challenge with *Vibrio parahaemolyticus*. Values are the mean ± SE; bars with different letters (a, b, and c) differ significantly at $P<0.05$. Uppercase letters (A and B) indicated a significant difference at $P<0.05$ between pre- and post-challenge with *V. parahaemolyticus*

diets supplemented with *Ocimum* leaf extract showed significantly higher ($P<0.05$) IgM levels compared with the control group under both pre- and post-challenge conditions. Among the analyzed tissues, IgM activity was highest in the skin, followed by the kidney, and lowest in the spleen. Prior to challenge, the highest IgM levels were recorded in fish fed 4 g kg^{-1} *Ocimum* leaf extract in the skin, 16 g kg^{-1} in the spleen, and 8 g kg^{-1} in the kidney. Following challenge with *V. parahaemolyticus*, IgM levels increased further in all tissues, reaching peak values in fish fed 16 g kg^{-1} *Ocimum* leaf extract. Significant differences ($P<0.05$) in IgM levels were observed between the treatment groups and the control following bacterial challenge.

The interleukin-1β (IL-1β) levels in the spleen,

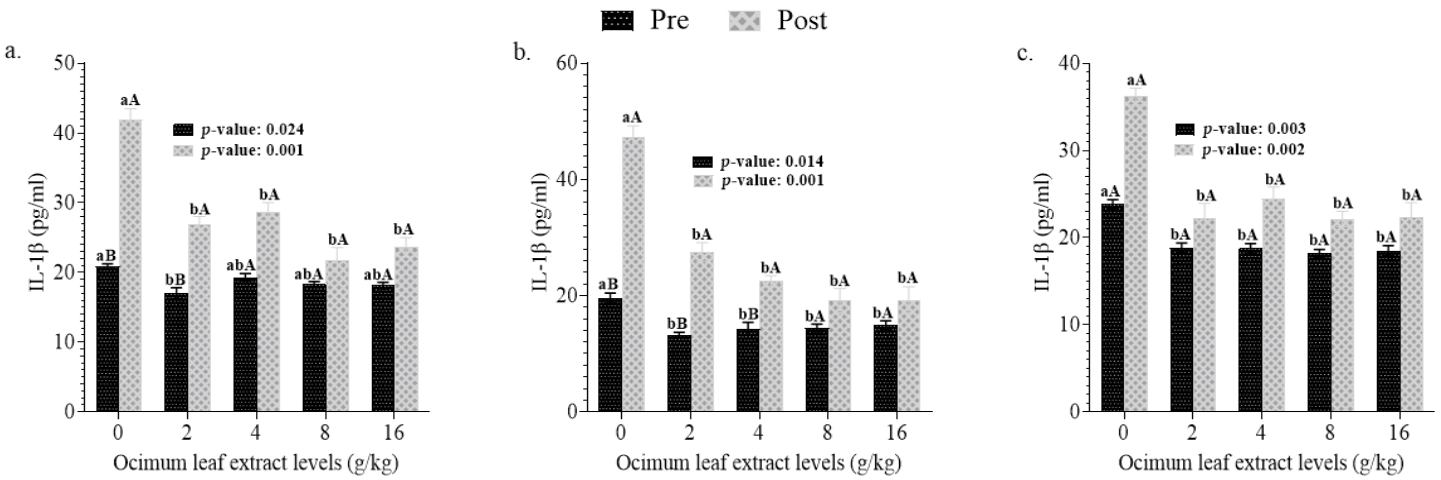

Figure 7. Interleukine-1β (IL-1β) activity in (a) spleen, (b) skin, and (c) kidney of *Oreochromis niloticus* fed a diet with different levels of *Ocimum gratissimum* leaf extract pre- and post-challenge with *Vibrio parahaemolyticus*. Values are the mean ± SE; bars with different letters (a, b, and c) differ significantly at $P<0.05$. Uppercase letters (A and B) indicated a significant difference at $P<0.05$ between pre- and post-challenge with *V. parahaemolyticus*

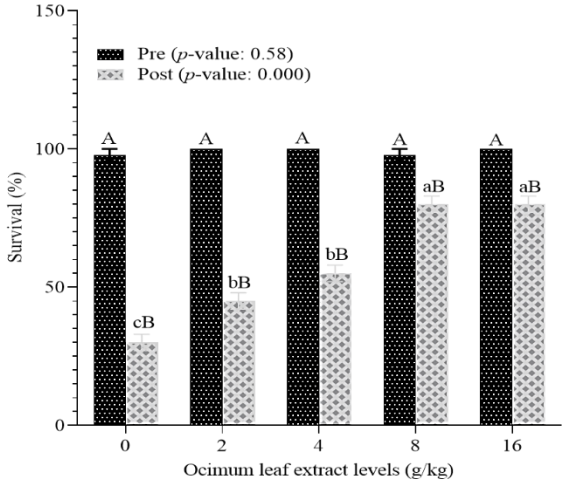

Figure 8. Percentage survival of *Oreochromis niloticus* fed different levels of *Ocimum gratissimum* leaf extract in the diet for 90 days and challenged with *Vibrio parahaemolyticus*. Values are mean ± SE; bars with different letters (a, b, and c) differ significantly at $P<0.05$. Uppercase letters (A and B) indicated a significant difference at $P<0.05$ between pre- and post-challenge with *V. parahaemolyticus*

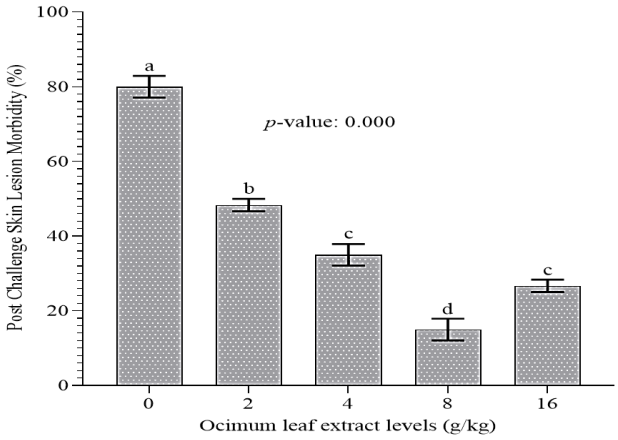

Figure 9. Skin lesion morbidity (%) of Oreochromis niloticus fed different levels of *Ocimum gratissimum* leaf extract challenged with *Vibrio parahaemolyticus*. Values are the mean ± SE; bars with different letters (a, b, and c) differ significantly at $P<0.05$

Skin and kidney tissues of *O. niloticus* are shown in Figure 7. The control group exhibited significantly higher IL-1β levels ($P<0.05$) than fish fed *Ocimum*-supplemented diets under both pre- and post-challenge conditions. Among the examined tissues, IL-1β levels were highest in the skin, followed by the spleen, and lowest in the kidney. Following challenge with *V. parahaemolyticus*, a significant increase ($P<0.05$) in IL-1β levels was observed in all tissues of the control group. Fish fed 2 g kg^{-1} *Ocimum* leaf extract showed a significant increase ($P<0.05$) in IL-1β levels in the spleen and skin after challenge, whereas fish fed 4 g kg^{-1} exhibited a significant increase only in the skin. In contrast, fish fed 8 and 16 g kg^{-1} *Ocimum* leaf extract showed no significant differences ($P>0.05$) in IL-1β levels between pre- and post-challenge conditions, indicating a potential modulatory effect of the extract on inflammatory responses.

Challenge test: No significant differences ($P>0.05$) in survival rate were observed among the experimental groups and the control group following the 90-day feeding trial prior to bacterial challenge (Fig. 8). However, after challenge with *V. parahaemolyticus*,

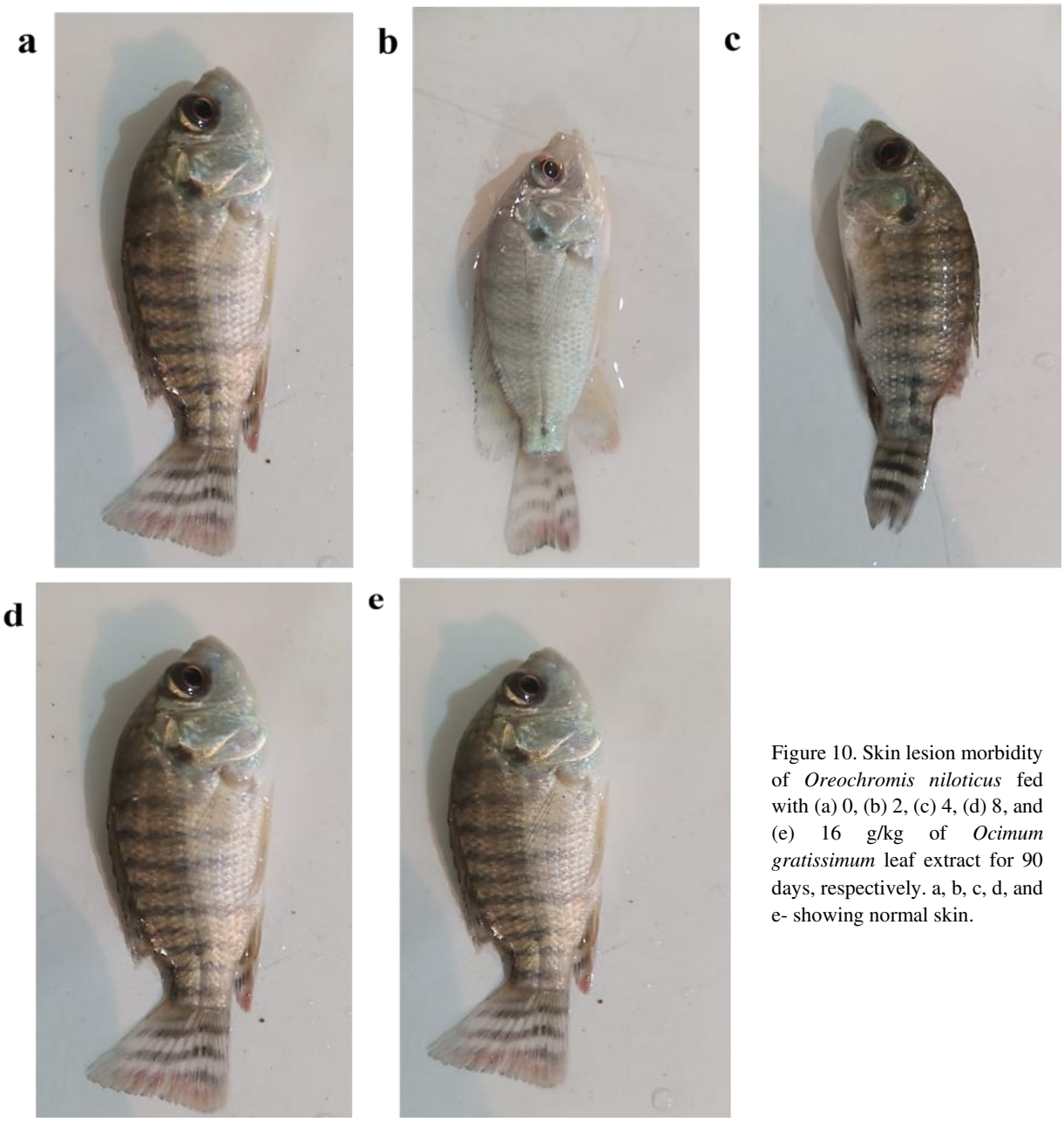

Figure 10. Skin lesion morbidity of *Oreochromis niloticus* fed with (a) 0, (b) 2, (c) 4, (d) 8, and (e) 16 g/kg of *Ocimum gratissimum* leaf extract for 90 days, respectively. a, b, c, d, and e- showing normal skin.

fish fed diets supplemented with *O. gratissimum* leaf extract exhibited higher survival rates compared with the control group. The highest post-challenge survival rate (80%) was recorded in fish fed diets containing 8 and 16 g kg^{-1} *Ocimum* leaf extract, whereas the lowest survival rate (30%) was observed in the control group receiving no dietary supplementation.

The severity of skin lesion morbidity following bacterial challenge is presented in Figures 9-11. Fish in the control group exhibited the highest percentage of skin lesion morbidity, whereas fish fed Ocimum-supplemented diets showed markedly reduced lesion severity. The highest skin lesion morbidity (80%) was recorded in the control group, while the lowest value (15%) was observed in fish fed 8 g kg^{-1} *Ocimum* leaf extract, indicating improved resistance against *V. parahaemolyticus* infection in the supplemented groups.

Discussions

The present study demonstrated that dietary supplementation with *O. gratissimum* leaf extract improved the hematological status and immune responsiveness of *O. niloticus*, particularly at the inclusion level of 8 g kg^{-1}. Fish fed *Ocimum*-supplemented diets exhibited significantly higher

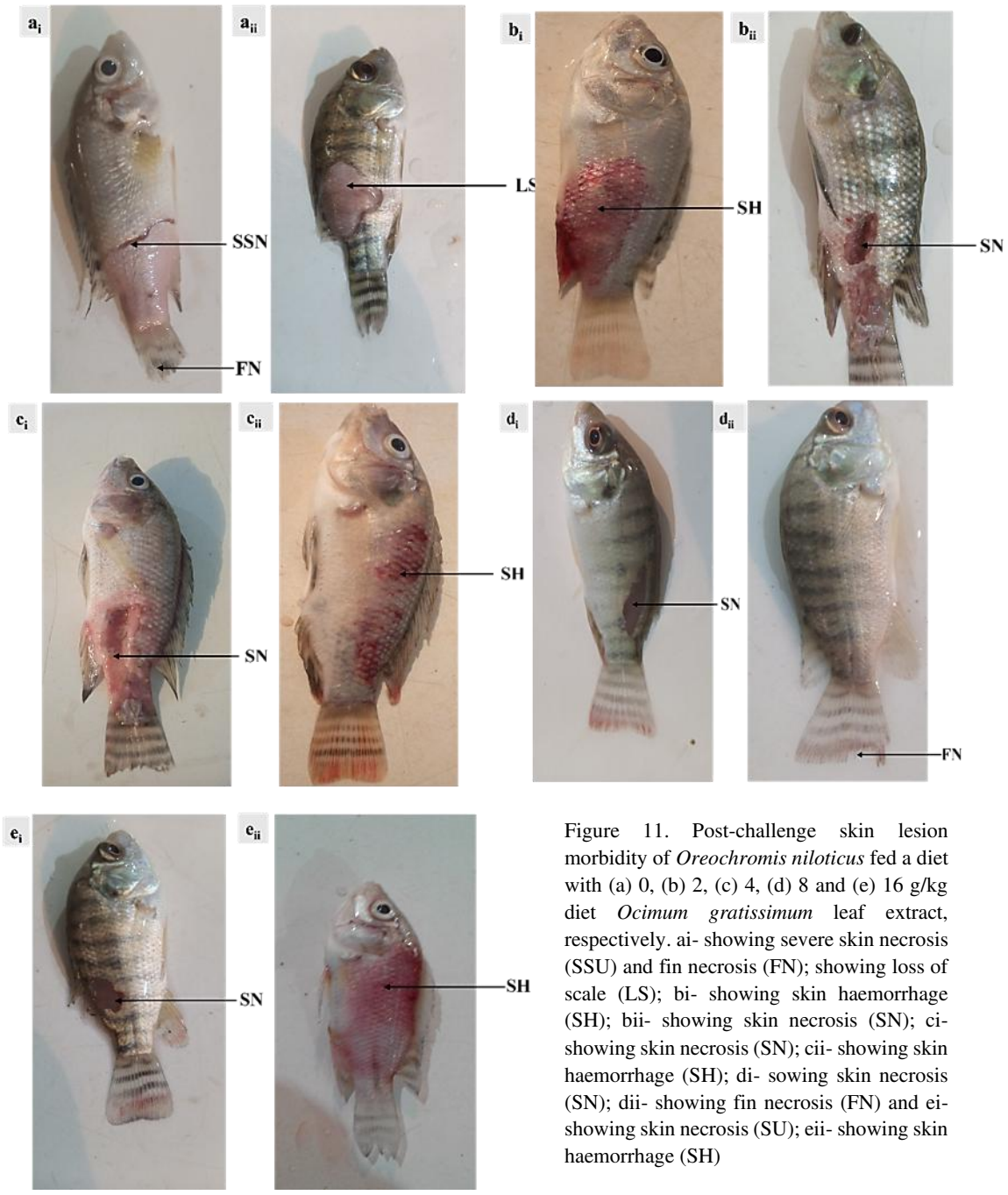

Figure 11. Post-challenge skin lesion morbidity of *Oreochromis niloticus* fed a diet with (a) 0, (b) 2, (c) 4, (d) 8 and (e) 16 g/kg diet *Ocimum gratissimum* leaf extract, respectively. ai- showing severe skin necrosis (SSU) and fin necrosis (FN); showing loss of scale (LS); bi- showing skin haemorrhage (SH); bii- showing skin necrosis (SN); ci- showing skin necrosis (SN); cii- showing skin haemorrhage (SH); di- sowing skin necrosis (SN); dii- showing fin necrosis (FN) and ei- showing skin necrosis (SU); eii- showing skin haemorrhage (SH)

WBC, RBC, HGB, and PCV values compared with the control group under both pre- and post-challenge conditions. In contrast, the control group showed a marked reduction in HGB concentration following *V. parahaemolyticus* challenge, indicating greater physiological stress and susceptibility to infection. However, no significant differences were observed in MCV and MCH among the experimental groups. The increased WBC counts observed in fish fed *Ocimum*-supplemented diets suggest enhanced nonspecific immune responses and improved defense mechanisms against pathogenic infection. Similar findings have been reported in *Clarias gariepinus*, rainbow trout (*Oncorhynchus mykiss*), and common carp (*Cyprinus carpio*) fed diets supplemented with phytogenic additives, including *Carica papaya* (paw-paw) aqueous leaf extract (Oparaku et al., 2024), *Plantago major* leaf extract (Khanzadeh et al., 2026), and papaya leaf extract (Giri et al., 2026).

The elevation of WBC levels after bacterial challenge further indicates immune system activation in response to *V. parahaemolyticus* infection.

Likewise, the higher RBC and HGB values observed in the supplemented groups indicate improved erythropoietic activity and oxygen-carrying capacity, reflecting better physiological condition and stress tolerance. Comparable improvements in RBC and HGB levels have also been reported in fish fed medicinal plant-based diets containing *Terminalia arjuna* bark (Nandi et al., 2026), spirulina and black cumin (Ayesha et al., 2026), and *Hunteria umbellata* (Jimoh et al., 2026). The reduction in HGB observed in the control group after bacterial challenge may be associated with hemolysis, impaired erythropoiesis, or infection-induced stress, whereas fish fed *Ocimum*-supplemented diets maintained relatively stable HGB levels, suggesting a protective effect against hematological disturbances. The lack of significant changes in MCV and MCH indicates that *Ocimum* leaf extract mainly influenced blood cell production rather than erythrocyte morphology. The beneficial effects observed in the present study are likely attributable to the rich phytochemical composition of *O. gratissimum*, particularly flavonoids, phenolics, thymol, and eugenol, which possess antioxidant and immunostimulatory properties that can enhance hematopoiesis, reduce oxidative stress, and stimulate immune-related pathways.

The present study demonstrated that dietary supplementation with *O. gratissimum* leaf extract improved the serum biochemical profile and hepatic health status of *O. niloticus*, particularly following *V. parahaemolyticus* challenge. Fish fed *Ocimum*-supplemented diets exhibited significantly lower ALT, AST, and glucose levels, whereas ALP, total protein, globulin, and albumin concentrations were significantly elevated compared with the control group. In contrast, the marked increase in ALT and glucose levels observed in the control group after bacterial challenge suggests greater physiological stress and hepatic impairment. Similar improvements in serum biochemical parameters have been reported in fish fed phytogenic additives, including *C. papaya* aqueous leaf extract (Oparaku et al., 2024), *P. major* leaf extract (Khanzadeh et al., 2026), papaya leaf extract (Giri et al., 2026), and fish mint (*Houttuynia cordata* Thunb.) (Yu et al., 2026). Elevated ALT and AST activities are commonly recognized as indicators of hepatocellular damage and physiological stress; thus, the lower enzyme activities observed in the supplemented groups suggest hepatoprotective effects of *O. gratissimum* (Refaey et al., 2023). Likewise, the reduced glucose levels in fish fed *Ocimum*-supplemented diets may reflect improved stress tolerance and metabolic regulation during bacterial infection. The increased concentrations of total protein, globulin, and albumin further indicate enhanced protein synthesis, immune competence, and overall physiological status (Reshi et al., 2023). These beneficial effects are likely attributed to the rich phytochemical composition of *O. gratissimum*, particularly flavonoids, phenolics, thymol, and eugenol, which possess antioxidant, antimicrobial, and hepatoprotective activities capable of mitigating oxidative damage and improving liver function in challenged fish (Silva et al., 2022; Ugbogu et al., 2021).

The present study showed that dietary supplementation with *O. gratissimum* leaf extract enhanced the antioxidant defense system of *O. niloticus*, as evidenced by increased SOD, catalase CAT, and reduced GSH activities, together with reduced MDA levels under both pre- and post-challenge conditions. The strongest antioxidant responses were generally observed in fish fed 8 g kg^{-1} *Ocimum* leaf extract, whereas the control group exhibited lower antioxidant enzyme activities and higher oxidative stress indicators following *V. parahaemolyticus* challenge. Similar improvements in antioxidant biomarkers have been reported in fish fed phytogenic additives such as *Ocimum* species (Abdel-Tawwab et al., 2018), *Perilla frutescens*, *Illicium verum*, and *Artemisia annua* extracts (Yue et al., 2024), Scote pine (*Pinus sylvestris*) essential oil (Yousefi et al., 2025), and liposome encapsulating pine bark extract (Ibrahim et al., 2025). Antioxidant enzymes, including SOD and CAT, play essential roles in protecting cells against reactive oxygen species (ROS) and oxidative damage (Hoseinifar et al., 2020; Khan et al., 2025). Therefore,

the elevated SOD and CAT activities observed in the supplemented groups indicate enhanced antioxidant defense capacity and improved protection against infection-induced oxidative stress (Jomova et al., 2024). Likewise, the higher GSH levels further support the antioxidative potential of *O. gratissimum*, whereas the reduction in GSH observed in the control group after bacterial challenge suggests depletion of antioxidant reserves due to excessive oxidative stress. MDA, a marker of lipid peroxidation and cellular membrane damage (Rizzo, 2024), was significantly reduced in fish fed *Ocimum*-supplemented diets, indicating lower oxidative damage and improved membrane stability. These beneficial effects are likely associated with the rich phytochemical composition of *O. gratissimum*, particularly flavonoids, phenolics, thymol, and eugenol, which possess strong antioxidant and free radical scavenging activities capable of enhancing endogenous antioxidant defenses and mitigating oxidative damage during bacterial infection (Akinmoladun et al., 2007; Mahapatra and Roy, 2014; Ballester et al., 2025).

The results of the current work revealed that dietary supplementation with *O. gratissimum* leaf extract enhanced the immune responses of *O. niloticus*, as evidenced by increased lysozyme and IgM activities and reduced IL-1β levels under both pre- and post-challenge conditions. Fish fed *Ocimum*-supplemented diets exhibited significantly higher lysozyme and IgM levels in the spleen, skin, and kidney compared with the control group, whereas IL-1β levels were significantly lower, particularly at 8 and 16 g kg^{-1} inclusion levels. These findings suggest that dietary *O. gratissimum* enhanced both innate and adaptive immune responses and modulated excessive inflammatory responses induced by *V. parahaemolyticus* infection. Similar immune-stimulatory effects have been reported in Nile tilapia and other fish species fed phytogenic additives, including *Ocimum* species, garlic, ginger, and medicinal herb extracts (Abdel-Tawwab et al., 2018; Ergena et al., 2023; Omitoyin et al., 2019) and *Avicennia marina* leaf extract (El Basuini, 2025). Lysozyme is an important component of the innate immune system involved in bacterial cell wall degradation and pathogen elimination (Mokhtar et al., 2023). Therefore, the elevated lysozyme activity observed in the supplemented groups indicates enhanced nonspecific immune defense. Likewise, the increased IgM levels suggest improved humoral immunity and antibody production (Huang et al., 2025). In contrast, the elevated IL-1β levels observed in the control group after bacterial challenge indicate stronger inflammatory responses associated with infection-induced stress and tissue damage. The lower IL-1β levels recorded in fish fed *Ocimum*-supplemented diets suggest anti-inflammatory effects and better regulation of immune responses during infection (Mansour et al., 2023). These beneficial effects are likely associated with the rich phytochemical composition of *O. gratissimum*, particularly flavonoids, phenolics, thymol, and eugenol, which possess antimicrobial, antioxidant, immunostimulatory, and anti-inflammatory properties that enhance immune competence and disease resistance in Nile tilapia.

The challenge test demonstrated that dietary supplementation with *O. gratissimum* leaf extract enhanced *O. niloticus* resistance to *V. parahaemolyticus* infection, as evidenced by higher post-challenge survival rates and lower skin lesion severity in the supplemented groups compared with the control group. Fish fed diets containing 8 and 16 g kg^{-1} *Ocimum* leaf extract showed the highest survival rate (80%), whereas the control group exhibited the lowest survival (30%). In addition, skin lesion morbidity was markedly reduced in fish receiving *Ocimum*-supplemented diets, particularly at 8 g kg^{-1} inclusion level. Similar improvements in disease resistance and survival have been reported in fish fed phytogenic additives such as *Gentiana Macrophylla* pall aqueous extract (Zhou et al., 2024), herbal extracts (Zhu et al., 2024), herbal extract blend (Zeng et al., 2025), papaya leaf extract (Giri et al., 2026), and α-mangostin-rich extract nanoemulsion (Srisaen et al., 2026). The lower survival and higher lesion severity observed in the control group may be associated with increased bacterial colonization, oxidative stress, and

weakened immune responses following infection. In contrast, the improved survival and reduced lesion formation in the supplemented groups were likely related to enhanced hematological, antioxidant, and immune responses induced by *O. gratissimum*. The protective effects of *O. gratissimum* are likely attributable to its rich phytochemical composition, particularly flavonoids, phenolics, thymol, and eugenol, which possess antimicrobial, antioxidant, anti-inflammatory, and immunostimulatory properties that enhance disease resistance and physiological resilience in Nile tilapia.

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