



Effects of Dietary Supplementation with *Halimeda opuntia* Powder on Growth Performance, Digestibility, Immune Response, Antioxidant Defensive System and Gene Regulation of *Oreochromis niloticus*

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Abstract

This study evaluated the impacts of dietary *Halimeda opuntia* (Chlorophyceae) powder on the growth performance, digestive enzyme activities, immune responses, antioxidant status, gene expression, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). A total of 200 fingerlings with an initial average weight of 10.35 ± 0.01 g were randomly allocated into four treatment groups: control, 0.25%, 0.5%, and 1.0% *Halimeda* powder. Each group was replicated across 5 aquaria, with 10 fish per aquarium. After 8 weeks, the 0.25% *Halimeda* group (H0.25) showed significant (linear, quadratic, and cubic trends) improvements in growth metrics such as final weight, weight gain, daily weight gain, weight gain percentage, and specific growth rate, compared to the control group. For digestive enzyme activities, the H0.25 group exhibited significantly higher amylase, pepsin, and protease activities, with a cubic response pattern ($P \leq 0.01$ or 0.001). Conversely, no significant linear or quadratic effects were observed for triglyceride levels. Meanwhile, the H0.25 group showed significantly higher total protein, albumin, and globulin levels, with cubic trends ($P \leq 0.05$ or 0.01), and declined liver enzyme activities (aspartate aminotransferase, AST and alanine aminotransferase, ALT) followed quadratic ($P \leq 0.001$ for AST, $P \leq 0.01$ for ALT) and cubic trends ($P \leq 0.01$ for AST, $P \leq 0.05$ for ALT). Immune responses, including lysozyme activity, interleukin-1-beta (IL-1 β), and tumor necrosis factor alpha (TNF- α) were significantly (linear, quadratic, and cubic trends) influenced by the supplementation, with the most notable effects observed in the H0.25 group. Regarding antioxidant status, glutathione (GSH) and superoxide dismutase (SOD) activities were significantly increased in the H0.25 group, following quadratic and cubic trends ($P \leq 0.05$ for GSH, $P \leq 0.001$ for SOD). Malondialdehyde (MDA) levels were significantly reduced, showing quadratic and cubic trends ($P \leq 0.001$). Gene expression analysis revealed upregulation of both Proliferating Cell Nuclear Antigen (PCNA) and SOD genes in the liver specimens of the 0.25% treated group, with significant linear and quadratic trends ($P \leq 0.01$ for both PCNA and SOD). Meanwhile, histological examination indicated enhanced intestinal morphology, with elongated villi and improved absorptive capacity in combination with the dietary administration. In conclusion, a 0.25% inclusion of *H. opuntia* powder optimizes growth, immune function, antioxidant status, and intestinal health, with significant dose-dependent influences.

Keywords Growth · *Halimeda opuntia* · Antioxidant · *Oreochromis niloticus* · Gene Expression

Introduction

Aquaculture has emerged as a vital sector in global food production, with Nile tilapia (*Oreochromis niloticus*) playing a central role due to its fast growth, high feed conversion efficiency, and adaptability to various environmental conditions (Elbially et al. 2022). However, intensive aquaculture systems often expose fish to multiple stressors, including pathogens,

suboptimal water quality criteria, and metabolism imbalances, which may suppress their growth, immunity, and overall health (Negm et al. 2021). Therefore, there is a growing interest in functional feed additives that can sustainably enhance fish performance and resilience (Naiel et al. 2023). Among these, natural alternatives to antibiotics, particularly those derived from marine sources, have received increasing attention for their ability to support health without contributing to antimicrobial resistance (Naiel et al. 2021).

In this context, marine algae have emerged as particularly promising functional ingredients, offering a wide range of

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bioactive compounds that can promote aquaculture production. Several studies have highlighted the benefits of incorporating different marine algae species into aquafeeds to improve fish health and performance (Villar-Navarro et al. 2021). For example, supplementation with *Ulva lactuca* and *Gracilaria* spp. has been associated with enhanced growth rates, improved intestinal histomorphology, and strengthened immune responses in various fish species, including tilapia (Suryaningrum et al. 2017) and seabass (Passos et al. 2021). Brown macroalgae such as *Ascophyllum nodosum* and *Sargassum* spp. have demonstrated potent antioxidant and anti-inflammatory properties, largely attributed to their high content of phlorotannins and fucoidans (Oliveira et al. 2014; Negm et al. 2021). These compounds help mitigate oxidative stress and support immune system modulation under intensive farming conditions (Gisbert et al. 2023). Additionally, red algae like *Porphyra* and *Chondrus crispus* have been reported to promote mucosal health and disease resistance (Guerreiro et al. 2019; Siddik et al. 2023). Collectively, these findings suggest that marine algae represent valuable bioresources for sustainable aquafeed formulations, though the specific effects and optimal inclusion levels often vary depending on algal species, processing method, and fish type (Sheikhzadeh et al. 2022).

Among the less commonly explored but highly promising algae species is *Halimeda opuntia*, a green calcareous macroalga with distinct bio-functional properties (Gomez-Zavaglia et al. 2019). It contains various bioactive constituents such as polysaccharides, terpenoids, phenolic compounds, flavonoids, saponins, alkaloids, and pigments like chlorophyll and carotenoids (Saber et al. 2025). These compounds exhibit numerous biological activities, including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects (Darfiah et al. 2021). For instance, terpenoids and flavonoids are known to scavenge free radicals and protect cellular structures from oxidative damage, while polysaccharides can enhance immune responses by activating macrophages and modulating cytokine production (Khan et al. 2018; Yahfoufi et al. 2018; Alarabei et al. 2023; Khanzadeh et al. 2025). Such multifunctional properties highlight *H. opuntia* as a promising feed additive in aquaculture (Van Doan et al. 2023).

Despite its therapeutic potential, no previous research has explored the incorporation of *Halimeda opuntia* into aquafeeds and its health and physiological impacts on fish, particularly Nile tilapia. Therefore, this study was conducted to evaluate the impacts of dietary supplementation with *H. opuntia* powder on growth performance, digestive enzyme activity, immune responses, antioxidant defensive features, gene expression, and intestinal morphology in Nile tilapia. By elucidating these effects, the current research not only aims to establish the optimal inclusion level of *H. opuntia* for promoting health and productivity in cultured fish but also introduces a novel and sustainable macroalgal as a functional

feed additive with high practical potential for enhancing fish resilience in intensive aquaculture systems.

Materials and Methods

Algae Collection and Preparation Procedure

Halimeda opuntia was collected during low tide from the rocky intertidal zone (27°17'13"N; 33°46'21"E) along the Red Sea shore at Hurghada, Egypt. At the collection site, algae specimens were initially rinsed twice with clean seawater to remove surface impurities such as epiphytes and debris, then transported to the laboratory in clean plastic bags. In the lab, samples were thoroughly washed with freshwater and repeatedly rinsed with distilled water to eliminate any remaining contaminants. The identity of *H. opuntia* was confirmed through morphological identification by a phycology specialist, following standard taxonomic keys (Khanzadeh et al. 2025). The cleaned *H. opuntia* samples were then spread indoors and air-dried at 22 ± 1.5 °C for 5 days. After drying the algae were finely ground using an electric grinder, and the prepared powder was stored in sealed containers at 4 °C until further use.

Fish Welfare Conditions and Experimental Design

This feeding trial was conducted in the wet laboratory of the Fish Nutrition and Technology Department at the Central Laboratory for Aquaculture Research (CLAR) in Abo-Hammad, Al-Sharqia, Egypt. A total of 200 Nile tilapia (*Oreochromis niloticus*) fingerlings, with an initial average weight of 10.35 ± 0.01 g, were obtained from the CLAR freshwater hatchery in Abbassa. The fish were randomly distributed into four treatment groups, each replicated across five glass aquaria (100 L capacity each), with 10 fish per aquarium. The control group was offered a basal diet without additives, while the other three groups received diets supplemented with *H. opuntia* powder at inclusion levels of 0.25%, 0.5%, and 1.0%. The inclusion levels of *H. opuntia* were chosen based on the dosages reported by Moutinho et al. (2018). 30% of the water in each aquarium was replaced daily with dechlorinated tap water to maintain the water quality criteria within desirable levels. Aeration was continuously supplied using air stones connected to electrical air pumps. Throughout the experimental period, water temperature was maintained at 27 ± 1 °C, dissolved oxygen levels were kept above 5 mg L^{-1} , and pH was stabilized within the range of 7.2–7.8. The photoperiod was set at 12 h light and 12 h dark. Prior to the feeding trial experiment, fish were acclimatized for two weeks on a standard 30% protein diet under laboratory conditions. The experimental diets were carefully formulated to satisfy the nutritional requirements of Nile tilapia. All dietary ingredients were thoroughly mixed with an appropriate amount of water to achieve a consistent dough-like texture. The mixture

was then passed through a pellet machine to produce 1.5 mm diameter feed pellets. To ensure cohesion and prevent disintegration of the pellets in water, a small amount of starch was added as a natural binder during the mixing process. The pellets were dried at 25 °C for approximately 48 h in a well-ventilated room until they reached a stable moisture content. Once dried, the pellets were stored in airtight containers at 4 °C until use. Detailed formulations and proximate compositions of the diets and *H. opuntia* powder are presented in Table 1. Throughout the 8-week trial, fish were fed twice daily to apparent satiation.

Growth and Efficiency of Consumed Feed Indices

During the 8-week feeding trial fish were provided with their respective diets and weighed biweekly to monitor feed consumption and assess growth performance. The feeding rate was adjusted every two weeks based on the average body weight of the fish in each group, ensuring that feed was offered at a fixed percentage of biomass (5% of body weight per day). This approach allowed for accurate rationing and

minimized feed waste while supporting optimal growth. The following parameters were used to evaluate growth:

Weight Gain (WG, g) = Final body weight – Initial body weight

Daily Weight Gain (DWG, g day⁻¹) = WG / Number of trial days

Weight Gain Percentage (WG%) = (WG / Initial weight) × 100

Specific Growth Rate (SGR, %day⁻¹) = [Ln(Final weight)

– Ln (Initial weight)] / Number of trial days

Utilization of consumed diet was assessed through:

Total Feed Intake (TFI) (fish⁻¹) = Total feed consumed / Number of fish

Feed Conversion Ratio (FCR, g g⁻¹) = TFI / WG

Feed Efficiency (FE, g g⁻¹) = WG / TFI

Sampling Protocol

By the end of the 8-week feeding experiment all fish were fasted for 24 h prior to sampling. From each treatment group, 10 fish (2 individuals per aquarium) were randomly selected for sample collection. To minimize stress, the selected fish were anesthetized within 3 min using clove oil at a concentration of 95 mg L⁻¹ in accordance with the protocol described by Adeshina et al. (2016). Blood samples were collected from the caudal vein using 1 mL syringes. For biochemical analyses, samples were transferred into Eppendorf tubes containing dipotassium EDTA (0.5 mg mL⁻¹ blood) as an anticoagulant. For the assessment of antioxidant and immune biomarkers, separate samples were collected in heparinized tubes to avoid interference with enzymatic activity and ion-dependent reactions. These samples were left at room temperature for 30 min prior to centrifugation at 3000 ×g for 15 min to separate the serum, which was subsequently stored at –80 °C for later analysis of biochemical, antioxidant, and immune markers. Liver specimens were also collected from the same fish used for blood sampling and immediately preserved in liquid nitrogen at –70 °C to be used for gene expression analysis. Portions of the mid-intestine were carefully dissected and preserved in graded alcohol solutions for histopathological analysis, while a small fragment was used to assess digestive enzyme activity.

Serum Biochemical and Lipid Metabolism Indices

Total protein (TP) and albumin (ALB) levels in blood serum were measured using a colorimetric method employing commercial assay kits (Biodiagnostic, Giza, Egypt), while serum globulin (GB) was calculated by subtracting the albumin value from the total protein. Liver enzyme activity, including alanine transaminase (ALT) and aspartate transaminase (AST), was quantified using a 384-well colorimetric/fluorometric assay kit (Cat. No. ABACM241035, Abcam, UK), as outlined by Wilkinson et al. (1972). Kidney function indicators, such as

Table 1 The experimental diets composition and analysis

Ingredients	CNT	H _{0.25}	H _{0.5}	H _{1.0}
Fish meal	9	9	9	9
Soybean meal (46% CP)	24	24	24	24
Wheat bran	26.4	26.15	25.9	25.4
Corn	24.4	24.4	24.4	24.4
Corn gluten meal (60% CP)	10.3	10.3	10.3	10.3
Wheat meal	2.4	2.4	2.4	2.4
Plant oil	2	2	2	2
Fish oil	1	1	1	1
Vit & Min premix ¹	0.5	0.5	0.5	0.5
Halimeda algae powder	0.0	0.25	0.5	1
Total	100	100	100	100
Proximate analysis (% air-dry basis)				
NFE ³	51.42	51.53	51.52	51.44
GE (MJ kg ⁻¹ diet) ⁴	18.87	18.91	18.92	18.96
Crude protein (N × 6.25)	30.52	30.61	30.55	30.67
Ash	4.98	5.08	5.37	5.46
Lipids	7.19	7.17	7.24	7.32
Fiber	5.89	5.61	5.32	5.11

¹Composition of mineral premix kg⁻¹: Mg, 53 g; Zn, 40 g; Fe, 20 g; Cu, 2.7 g; I, 0.34 g; Se, 70 mg; Co, 70 mg and calcium carbonate as carrier up to 1 kg. vitamin A, 8000000 IU; vitamin D₃, 2000000 IU; vitamin E, 7000 mg; vitamin K₃, 1500 mg; vitamin B₁, 700 mg; vitamin B₂, 3500 mg; vitamin B₆, 1000 mg; vitamin B₁₂, 7 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20000 mg; pantothenic acid, 7000 mg.

³NFE (Nitrogen free extract) = (crude protein % + crude lipids % + ash % + crude fiber %) - 100.

⁴Gross energy (GE) was calculated from NRC (2011) as 23.6 kJ g⁻¹, 39.4 kJ g⁻¹, and kJ g⁻¹ for protein, lipid, and carbohydrates, respectively.

uric acid (UA) and creatinine (CR), were measured spectrophotometrically with specific diagnostic kits (BIOMED, Enzymatic/Colorimetric, Egypt), following Folin and Wu (1919). Meanwhile, triglyceride levels were estimated using the RA-50 Chemistry Analyzer (Bayer) and pre-prepared kits from Spin-react Co. (Spain), following the detailed outlined guidelines.

Immune and antioxidative function measurements

Serum lysozyme (LYZ) values were estimated by the lyophilized *Micrococcus lysodeikticus* cells (Sigma-Aldrich, USA) procedure. Each complete LYZ unit was equal to a reduction unit of 0.001 in optical density (OD) at 520 nm per min, as described by Ellis (1990). Absorbance readings were taken at the start (0 min) and after 5 min of incubation at room temperature to determine the rate of OD decline. While, the ACH₅₀ (alternative complement activity) in blood was determined using a hemolytic assay as described by Yano et al. (1988). Briefly, a 1:10 dilution of RaRBCs was prepared and incubated with fish serum in a Mg²⁺-EGTA-gelatin veronal buffer (GVB⁺⁺) at 22–25 °C for 90 min. The reaction was then stopped by adding cold saline, followed by centrifugation at 1500 × *g* for 10 min. The degree of haemolysis was assessed spectrophotometrically at 415 nm, and the ACH₅₀ value was calculated as the volume of serum causing 50% haemolysis of RaRBCs, expressed in units mL⁻¹.

Serum interleukin (IL-1β) and tumor necrosis factor (TNF-2α) were measured using the immunosorbent assay (ELISA) technique with specific commercial kits (Diacclone Kit, France) as described by Zhang et al. (2025). Briefly, 100 μL of standards and diluted serum samples were added to pre-coated 96-well microplates and incubated at 37 °C for 2 h. Following a series of wash steps, biotinylated detection antibodies and streptavidin–HRP conjugate were applied sequentially, and the colorimetric reaction was developed using TMB substrate. Absorbance was measured at 450 nm using a microplate reader, and cytokine concentrations were determined from the standard curves.

Serum levels of malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) activity were measured using commercial colorimetric assay kits (Bio-Diagnostic Co., Giza, Egypt). Specifically, MDA levels were assessed based on the thiobarbituric acid reactive substances (TBARS) method described by Draper and Hadley (1990). The pink MDA-TBA adduct was measured at 534 nm. GSH levels were quantified using the method of Beutler and Mary (1963), in which 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) reacts with sulfhydryl groups to form a yellow-colored complex measured at 405 nm. SOD activity was determined by the method of Nishikimi et al. (1972) based on the enzyme's ability to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals, with absorbance measured at 560 nm. All assays were performed in triplicate, and results expressed per mL of serum.

Determination of Digestive Enzyme Activities

Firstly, the mid-intestinal samples were homogenized in an ice-cold buffer solution consisting of 50 mM Tris-HCl (pH 7.5–8.0), 150 mM NaCl, 1–2 mM EDTA, and 0.1–0.5% Triton X-100, with a protease inhibitor cocktail to prevent protein degradation during processing. After homogenization, the prepared mixture was centrifuged at 10,000 × *g* for 10 min at 4 °C and the supernatant was stored at –80 °C for enzyme activity assays. Enzyme activities of amylase, lipase, and protease were quantified spectrophotometrically at A₅₄₀, A₇₁₄, and using casein as a substrate, respectively, as described by Carrière et al. (2005).

RNA Extraction, cDNA Synthesis, and Gene Expression Analysis

Total RNA was extracted from approximately 30 mg of liver tissues using the Eastep Super Total RNA Extraction Kit (Promega, Shanghai, China; Cat. No. LS1040), following the manufacturer's instructions. For each treatment group, liver tissue samples were collected from three fish per replicate (*n* = 3), with RNA individually extracted, resulting in a total of 9 biological samples per treatment group. To eliminate potential DNA contamination, 2 μL of the extracted RNA (10 mg mL⁻¹) was treated with DNase I in the presence of TE buffer (pH 8.0) and incubated at 37 °C for 3–4 hours. RNA purity and integrity were confirmed through 1.5% agarose gel electrophoresis and by measuring the A₂₆₀/A₂₈₀ ratio using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). First-strand cDNA was synthesized from 1 μg of total RNA using the GoScript Reverse Transcription Mix (Promega, China; Cat. No. LS2054), according to the manufacturer's protocol.

Quantitative real-time PCR (qRT-PCR) was conducted in a 20 μL reaction volume comprising 10 μL of 2× GoTaq qPCR Master Mix, 0.4 μL each of forward and reverse primers, 2 μL of template cDNA and 7.2 μL of nuclease-free water. Amplification conditions involved an initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A melting curve analysis from 60 °C to 95 °C was included to verify product specificity. Each sample was run in triplicate, including the reverse transcription step, to ensure result accuracy. The primer sequences used for amplifying the target genes included *HSP70* (heat shock protein 70), *Caspase-3* (a cysteine–aspartic acid protease), *PCNA* (proliferating cell nuclear antigen), *CAT* (catalase), *SOD* (superoxide dismutase), *GPx* (glutathione peroxidase), *GST* (glutathione-S-transferase), and *GSR* (glutathione reductase) along with the housekeeping genes *GAPDH* and *β-Actin* were designed using Primer-BLAST (NCBI), based on published gene sequences of Nile tilapia (*O. niloticus*) available in the NCBI GenBank database. All primer sets were evaluated for specificity, GC content, melting temperature, and potential secondary structures. The primers were synthesized by Macrogen (Korea) and

Table 2 The nucleotide sequences of applied designed primers used for *q*-RT-PCR applicable method of specific identified genes

Gene	Forward 5'→3'	Reverse 5'→3'	Accession NO.	T _m (°C)	T _a (°C)	Product Size (bp)	Efficiency (%)
<i>HSP70</i>	TTCAAGGTGATTTCAGAC GGAG	CTTCATCTTCACCAGGAC CATG	NC_031969.2	60.2	58	180	97.8
<i>Caspase-3</i>	GGCTCTTCGTCTGCTTCT GT	GGGAAATCGAGGCGGTATCT	NC_031966.2	59.5	58	150	98.3
<i>PCNA</i>	CCCTGGTGGTGGAGTACA AG	AGAAGCCTCCTCATCGAT CTTC	NC_031977.2	60.0	58	172	99.1
<i>CAT</i>	CCCAGCTCTTCATCCAGA AAC	GCCTCCGCATTGTACTTCTT	NC_031972.2	59.8	58	163	96.5
<i>SOD</i>	GGTGCCCTGGAGCCCTA	ATGCGAAGTCTTCCACTGTC	NC_031980.2	60.5	58	145	97.9
<i>GPx</i>	CGCCGAAGGTCTCGTTATT	TCCCTGGACGGACACTT	NC_031970.2	58.9	58	155	95.7
<i>GST</i>	AAATGGATGGCATGAAGC TC	TCGTCTTTGGGATCCTTTG	NC_031985.2	60.0	58	168	98.4
<i>GSR</i>	TACGCCAAATCTCCCACA AA	CCGACAGGTAAACACCGTAAT	NC_031966.2	59.1	58	162	96.8
<i>β-Actin</i>	TATGGCTAGAGCCAGGCA ACC	AATCCCAATAAAGTGACATG	XM_003443127	60.3	58	148	100.0
<i>GAPDH</i>	CCGATGTGTCAGTGGTGG AT	GCCTTCTTGACGGCTTCCTT	NM_001279552.1	60.1	58	160	99.2

HSP70: heat shock protein 70; *Caspase-3*: a cysteine–aspartic acid protease; *PCNA*: Proliferating cell nuclear antigen; *CAT*: catalase; *SOD*: superoxide dismutase; *GPx*: glutathione peroxidase; *GST*: glutathione-*s*-transferase; *GSR*: glutathione reductase; *β-Actin*, beta actin; *GAPDH*, Glyceraldehyde 3-phosphate dehydrogenase.

their sequences, amplicon sizes, and corresponding GenBank accession numbers are detailed in Table 2. Relative expression levels were normalized using *GAPDH* and *β-Actin* as internal reference genes. The fold change in gene expression was calculated using the $2^{\Delta\Delta C_t}$ method following Livak and Schmittgen (2001) calculation equation, after determining threshold cycle (C_t) values for all reactions.

Intestinal Histological Examination

Mid-intestinal samples ($n = 10$ per treated group) were carefully excised and immediately preserved in 10% buffered formalin for histological assessment. The fixed tissues were gradually dehydrated through an ascending ethanol series (70 to 100%), cleared in xylene, and embedded in paraffin wax. Using a rotary microtome (Leica RM 2155, England), tissue blocks were sectioned at a thickness of 5 μ m. These sections were then stained with hematoxylin and eosin (H&E), following the standard histological technique outlined by Kent et al. (1995). The stained slides were examined for histopathological alterations as described by Suvarna et al. (2018).

Statistical model

All recorded and computed data were statistically evaluated using a completely randomized design through SPSS software (version 22), employing the model: $Y_{ij} = \mu + T_i + E_{ij}$, where μ represents the overall mean, T_i is the fixed effect of

Halimeda algae dietary treatments, and E_{ij} denotes the random error. The Shapiro–Wilk test was used to confirm the normal distribution of the data, while Levene's test was applied to assess the homogeneity of variances among treatment groups prior to conducting parametric statistical analyses. Treatment effects were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test to determine significant differences among treatments. Polynomial trend analyses (linear, quadratic, and cubic) were performed to explore treatment response patterns. Gene expression results were visually displayed using GraphPad Prism version 9. Data are reported as mean values $\pm$ standard error (SE), and differences were considered statistically significant at $P < 0.05$.

Results

Growth and feed efficiency indices

Table 3 displays the effects of dietary supplementation with *Halimeda* algae powder (H) at different inclusion levels on fish growth and feed efficiency, compared to an un-supplemented group (CNT). Notably, the 0.25% *Halimeda* group (H0.25) exhibited significant improvements in final weight (FW), weight gain (WG), daily weight gain (DWG), weight gain percentage (WG%), and specific growth rate (SGR) compared to both the control group and the higher inclusion levels. These parameters demonstrated significant linear ($P \leq 0.05$),

Table 3 Effects of different dietary inclusion levels of *Halimeda* algae powder (H; 0.25, 0.5 and 1%) on growth rate and efficiency indices of consumed feed of *O. niloticus* for 8 weeks ($n = 200$)

Measurements	Treatments				P value		
	CNT	H _{0.25}	H _{0.5}	H _{1.0}	Linear	Quadratic	Cubic
IW, g	10.35±0.01	10.31±0.03	10.36±0.02	10.30±0.01	0.122	0.281	0.229
FW, g	37.75±0.36 ^b	39.86±0.45 ^a	38.11±0.06 ^b	36.70±0.75 ^b	0.018	0.015	0.017
WG, g	27.40±0.35 ^b	29.55±0.47 ^a	27.74±0.05 ^b	26.41±0.74 ^b	0.020	0.017	0.015
DWG, g day ⁻¹	0.49±0.01 ^b	0.53±0.21 ^a	0.50±0.14 ^b	0.47±0.11 ^b	0.021	0.018	0.014
WG%	264.8±3.32 ^b	286.7±5.28 ^a	267.7±1.44 ^b	256.5±6.94 ^b	0.029	0.022	0.010
TFI, g	43.36±0.80 ^b	48.60±1.14 ^a	46.84±1.03 ^{ab}	46.63±0.84 ^{ab}	0.156	0.019	0.033
FCR, g g ⁻¹	1.58±0.03	1.65±0.06	1.69±0.03	1.77±0.08	0.025	0.823	0.904
SGR, g day ⁻¹	2.31±0.20 ^b	2.41±0.22 ^a	2.33±0.11 ^b	2.27±0.14 ^b	0.029	0.023	0.013
FER, g g ⁻¹	0.63±0.11	0.61±0.12	0.59±0.22	0.57±0.10	0.023	0.721	0.947

IW, initial weight; FW, final weight; WG, weight gain; DWG, daily weight gain; WG%, weight gain percent; TFI, total consumed feed; FCR, feed conversion ratio; SGR, specific growth rate; FER, feed efficiency ratio. ^{a,b} and ^c Different superscript letters within the same row indicate a significant difference between treated and non-treated groups ($P < 0.05$). Data are presented as mean ± SE.

quadratic ($P \leq 0.05$), and cubic ($P \leq 0.05$ or 0.01) trends, indicating a dose-dependent response. Furthermore, total feed intake (TFI) was significantly higher in the H0.25 group, with quadratic ($P \leq 0.05$) and cubic ($P \leq 0.05$) effects observed. The feed conversion ratio (FCR) declined as the levels of *Halimeda* powder increased, showing a significant linear trend ($P \leq 0.05$), although quadratic and cubic responses were not significant. Additionally, the feed efficiency ratio (FER) significantly decreased with higher inclusion levels, displaying a linear trend ($P \leq 0.05$) without notable quadratic or cubic patterns. Overall, these findings suggest that a 0.25% inclusion of *Halimeda* powder (H0.25) optimizes growth and feed utilization, with several parameters reflecting linear, quadratic, and cubic dose-response relationships.

Digestive enzyme activities and lipid profile

Table 4 contents indicating the effects of *Halimeda* powder supplementation on digestive enzyme activities and triglyceride levels in the fish. The activities of digestive enzymes (amylase, AMY; pepsin, PEP; and protease, PROT) were significantly higher in the H0.25 group compared to the control (CNT) and other treated groups. These improvements

followed significant cubic trends ($P \leq 0.01$ for both AMY and PEP, and $P \leq 0.001$ for PROT), suggesting a non-linear response to the inclusion levels. However, no significant (linear or quadratic) effects were observed for these enzymes. Furthermore, triglyceride levels (TGLY) were statistically unaffected by dietary treatments. Overall, the results from Table 4 confirmed that dietary inclusion of *Halimeda* powder at a low inclusion level (0.25%) can promote digestive enzyme activities via a cubic response pattern, without significantly altering triglyceride concentrations.

Blood Biochemical indices

Fish fed the H0.25 diet exhibited significantly elevated concentrations of total protein (TP), albumin (ALB), and globulin (GLOB) compared to the control (CNT) (Table 5). These increases followed significant cubic trends ($P \leq 0.01$ for both TP and ALB, and $P \leq 0.05$ for GLOB) (Table 5). Additionally, hepatic enzyme activity was significantly reduced in the H0.25 group, pointing to enhanced liver function. These reductions aligned with pronounced quadratic ($P \leq 0.001$) and cubic ($P \leq 0.01$ for both AST and ALT) trends. Although uric acid (UA) levels were not significantly altered

Table 4 Effects of different dietary inclusion levels of *Halimeda* algae powder (H; 0.25, 0.5 and 1%) on digestive enzymes activities and lipid profile of *O. niloticus* for 8 weeks ($n = 10$).

Measurements	Treatments				P value		
	CNT	H _{0.25}	H _{0.5}	H _{1.0}	Linear	Quadratic	Cubic
AMY, U L ⁻¹	64.0±2.68 ^b	84.7±3.06 ^a	66.7±1.43 ^b	69.0±5.89 ^b	0.746	0.107	0.002
PEP, U L ⁻¹	11.7±1.25 ^b	20.0±0.82 ^a	13.3±0.85 ^b	13.7±2.32 ^b	0.760	0.063	0.002
PROT, ng L ⁻¹	0.06±0.11 ^b	0.08±0.42 ^a	0.06±0.32 ^b	0.06±0.22 ^b	0.753	0.203	0.001
TGLY, µmol L ⁻¹	94.1±3.27	90.6±1.39	96.9±1.69	99.1±5.49	0.173	0.710	0.279

AMY, amylase; PEP, pepsin; PROT, protease; TGLY, triglyceride. ^{a,b} and ^c Different superscript letters within the same row indicate a significant difference between treated and non-treated groups ($P < 0.05$). Data are mean ± SE.

Table 5 Effects of different dietary inclusion levels of *Halimeda* algae powder (H; 0.25, 0.5 and 1%) on serum protein constituents and liver and kidney function enzyme activities of *O. niloticus* for 8 weeks ($n = 10$)

Measurements	Treatments				P value		
	CNT	H _{0.25}	H _{0.5}	H _{1.0}	Linear	Quadratic	Cubic
TP, g dL ⁻¹	1.80±0.26 ^b	3.34±0.39 ^a	2.10±0.11 ^{ab}	2.12±0.46 ^{ab}	0.722	0.118	0.009
ALB, g dL ⁻¹	1.05±0.25 ^b	1.43±0.14 ^a	1.07±0.55 ^b	1.12±0.06 ^b	0.593	0.215	0.005
GLOB, g dL ⁻¹	0.75±0.24 ^b	1.91±0.27 ^a	1.03±0.06 ^{ab}	1.00±0.40 ^{ab}	0.786	0.122	0.017
AST, IU L ⁻¹	29.83±1.96 ^a	22.00±0.41 ^b	25.95±0.84 ^{ab}	28.65±0.39 ^a	0.505	0.001	0.004
ALT, IU L ⁻¹	18.62±0.28 ^a	14.82±0.24 ^b	16.03±0.62 ^b	16.62±0.64 ^b	0.132	0.001	0.006
UA, mg dL ⁻¹	0.64±0.10	0.37±0.02	0.42±0.12	0.64±0.13	0.586	0.017	0.374
CREA, mg dL ⁻¹	0.30±0.04 ^b	0.30±0.01 ^b	0.40±0.02 ^a	0.35±0.11 ^{ab}	0.064	0.076	0.040

TP, total protein; ALB, albumin; GLOB, globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; UA, uric acid; CREA, creatinine. ^{a,b and c} Different superscript letters within the same row indicate a significant difference between treated and non-treated groups ($P < 0.05$). data are mean ± SE.

by the treatments, a significant quadratic trend ($P \leq 0.05$) was observed. Creatinine (CREA) levels showed a significant increase in the H0.5 group relative to CNT and H0.25, accompanied by a significant cubic trend ($P \leq 0.05$), suggesting a possible dose-dependent effect.

Immune and antioxidant defensive parameters

Table 6 outlines the impact of dietary *Halimeda* algae powder on immune responses and oxidative stress biomarkers. Immune indicators such as lysozyme activity (LZM), interleukin-1 beta (IL-1 β), and tumor necrosis factor-alpha (TNF-2 α) were significantly influenced by the inclusion of *Halimeda*, particularly at 0.25%. The H0.25 group showed a marked increase in LZM and IL-1 β levels compared to the control (CNT), with significance supported by cubic trends ($P \leq 0.01$ for both). TNF-2 α levels significantly decreased across supplemented groups, with the lowest values in H0.25. This response followed significant linear ($P \leq 0.05$), quadratic ($P \leq 0.001$), and cubic ($P \leq 0.01$) trends.

However, Alternative complement activity (ACH50) was also highest in the H0.25 group, with significant quadratic and cubic trends ($P \leq 0.001$), suggesting enhanced innate immune response at this supplementation level. Regarding antioxidant status, the H0.25 group showed significantly elevated glutathione (GSH) and superoxide dismutase (SOD) activities, following quadratic ($P \leq 0.01$ for GSH and $P \leq 0.001$ for SOD) and cubic trends ($P \leq 0.001$ for GSH and $P \leq 0.001$ for SOD). Conversely, malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were significantly reduced in the H0.25 group, also showing quadratic and cubic trends ($P \leq 0.001$), indicating improved redox status balance.

Gene regulation

Figure 1 shows the hepatic gene expression profiles of *O. niloticus* after 56 days of feeding on diets enriched with *Halimeda* powder. The expression of HSP70 (heat shock protein 70) was not significantly influenced by dietary *Halimeda*

Table 6 Effects of different dietary inclusion levels of *Halimeda* algae powder (H; 0.25, 0.5 and 1%) on immune responses and antioxidant enzyme activities of *O. niloticus* for 8 weeks ($n = 10$)

Measurements	Treatments				P value		
	CNT	H _{0.25}	H _{0.5}	H _{1.0}	Linear	Quadratic	Cubic
Immune Activities							
LZM, U mL ⁻¹	6.30±0.86 ^b	10.90±0.63 ^a	6.33±0.75 ^b	7.07±1.37 ^b	0.551	0.231	0.003
IL-1 β , pg mL ⁻¹	6.06±0.64 ^b	8.54±0.19 ^a	6.24±0.34 ^b	6.74±0.65 ^b	0.841	0.221	0.003
TNF-2 α , pg mL ⁻¹	263.31±1.25 ^a	167.32±0.49 ^c	178.34±4.09 ^{bc}	208.31±1.84 ^b	0.020	≤0.001	0.007
ACH50, U mL ⁻¹	2.34±0.20 ^c	5.38±0.25 ^a	4.46±0.29 ^b	3.57±0.26 ^b	0.198	≤0.001	≤0.001
Redox Status							
GSH, mmol L ⁻¹	67.7±0.62 ^b	92.0±2.86 ^a	79.3±2.25 ^b	78.3±4.94 ^b	0.361	0.004	0.001
SOD, U mL ⁻¹	2.16±0.06 ^c	3.16±0.14 ^a	2.71±0.07 ^b	2.53±0.08 ^b	0.419	≤0.001	≤0.001
MDA, nmol mL ⁻¹	67.9±1.10 ^a	45.4±4.24 ^c	58.3±1.11 ^b	61.4±1.64 ^{ab}	0.977	0.001	≤0.001

LZM, lysozyme; IL-1 β , interleukin 1 beta; TNF-2 α , tumor necrosis 2 alpha factor; ACH50, alternative complement activities; GSH, glutathione; SOD, super oxide dismutase; MDA, malonaldehyde. ^{a,b and c} Different superscript letters within the same row indicate a significant difference between treated and non-treated groups ($P < 0.05$). data are mean ± SE

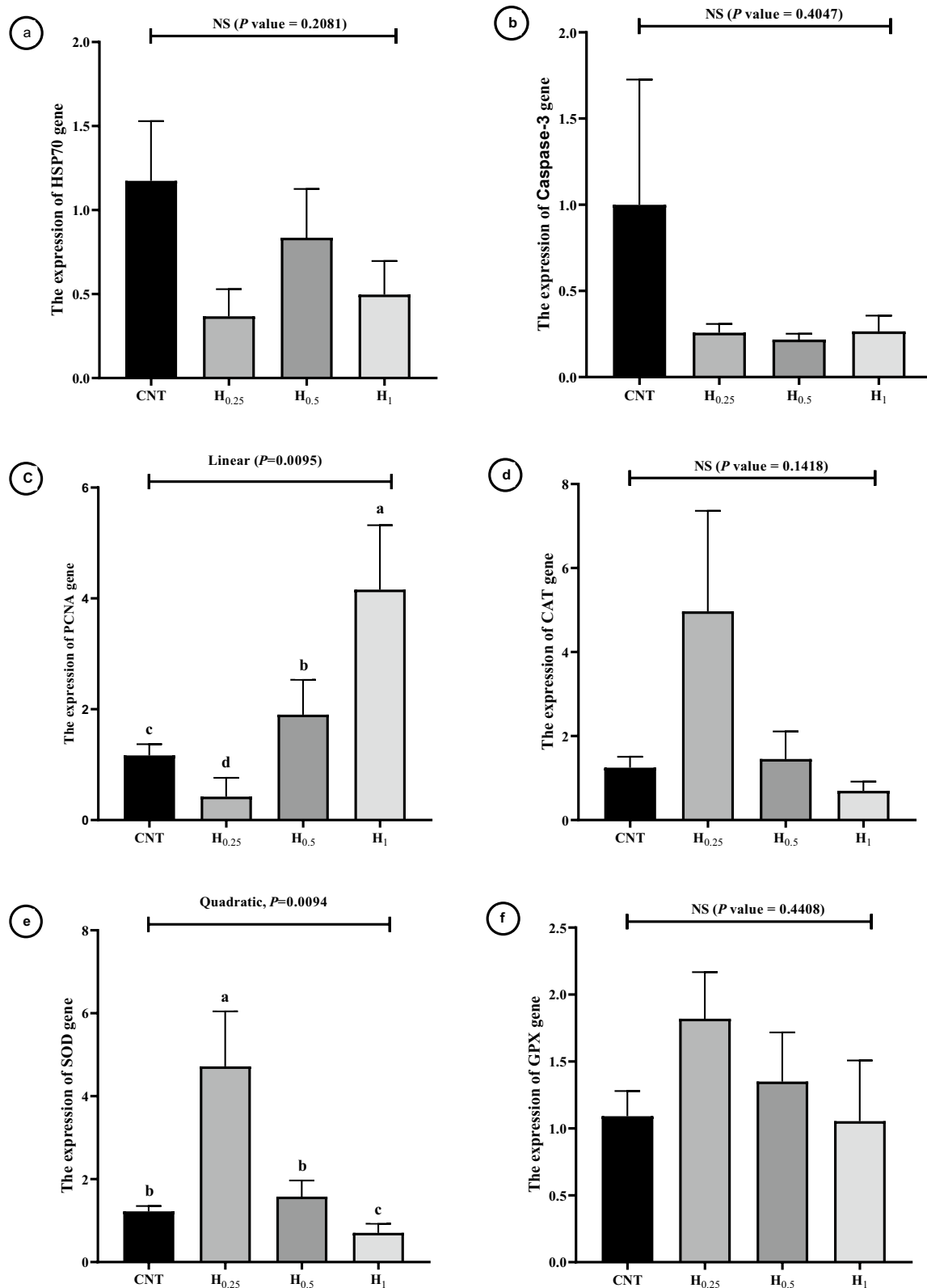


Fig. 1 The effect of *Halimeda* algae powder (0.25, 0.5 and 1%) supplemented diets on *HSP70*: heat shock protein 70 (a); *Caspase-3*: a cysteine–aspartic acid protease (b), *PCNA*: Proliferating cell nuclear antigen (c), *CAT*: catalase (d), *SOD*: superoxide dismutase (e), *GPx*:

glutathione peroxidase (f), *GST*: glutathione-s-transferase (g), and *GSR*: glutathione reductase (h) genes in livers of *O. niloticus* ($n = 10$). Bars indicate means $\pm$ S.E. ^{a,b} and ^c superscript letters within the bars indicate significant differences between groups ($P < 0.05$)

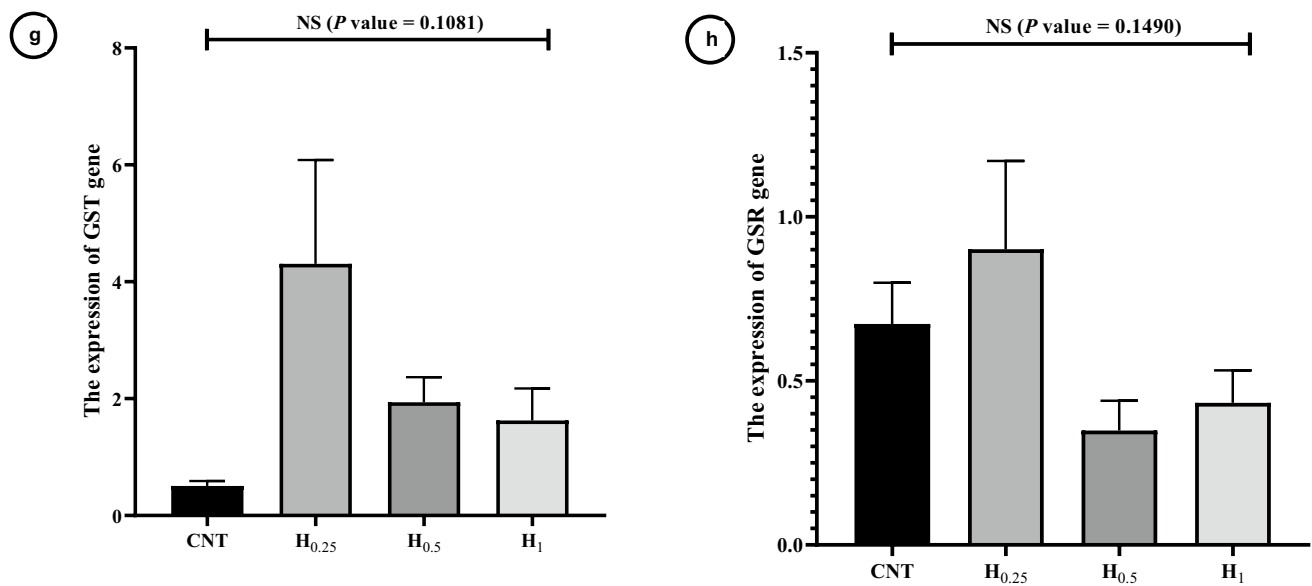


Fig. 1 (continued)

administration, nor was Caspase-3 (a cysteine–aspartic acid protease). Similarly, no significant changes were observed in CAT (catalase), GPx (glutathione peroxidase), GST (glutathione-S-transferase), or GSR (glutathione reductase) expression across treatment groups. In contrast, a significant linear increase in PCNA (proliferating cell nuclear antigen) expression was detected ($P \leq 0.01$), with the highest expression level recorded in fish receiving the 1% *Halimeda* diet compared to the control and lower inclusion levels. Additionally, SOD (superoxide dismutase) expression was significantly affected, displaying a quadratic trend ($P \leq 0.01$), with peak expression in the 0.25% group.

Histological examination

Figure 2 shows photomicrographs of intestinal sections from *Oreochromis niloticus* fed diets supplemented with varying levels of *Halimeda* powder for 56 days. In the control group (CNT), the intestinal tissue maintained a typical histological organization, with a clearly defined mucosal layer, intact submucosa, and a well-structured muscularis layer. Fish fed the 0.25% *Halimeda* diet (H0.25) had a preserved mucosal structure, along with noticeable branching at the tips of the intestinal villi, indicative of enhanced surface area. Furthermore, the intestinal architecture remained intact, with prominently broadened and well-formed villous ends, suggesting improved nutrient absorptive capacity in the 0.5% *Halimeda* group (H0.5). Fish in the 1% *Halimeda* group (H1.0) showed marked histological enhancements, including significantly elongated villi and a distinct corrugation of the epithelial surface. These morphological alterations imply a potential stimulation of mucosal development and an increase in the

digestive and absorptive efficiency in response to the inclusion level of *Halimeda* supplementation.

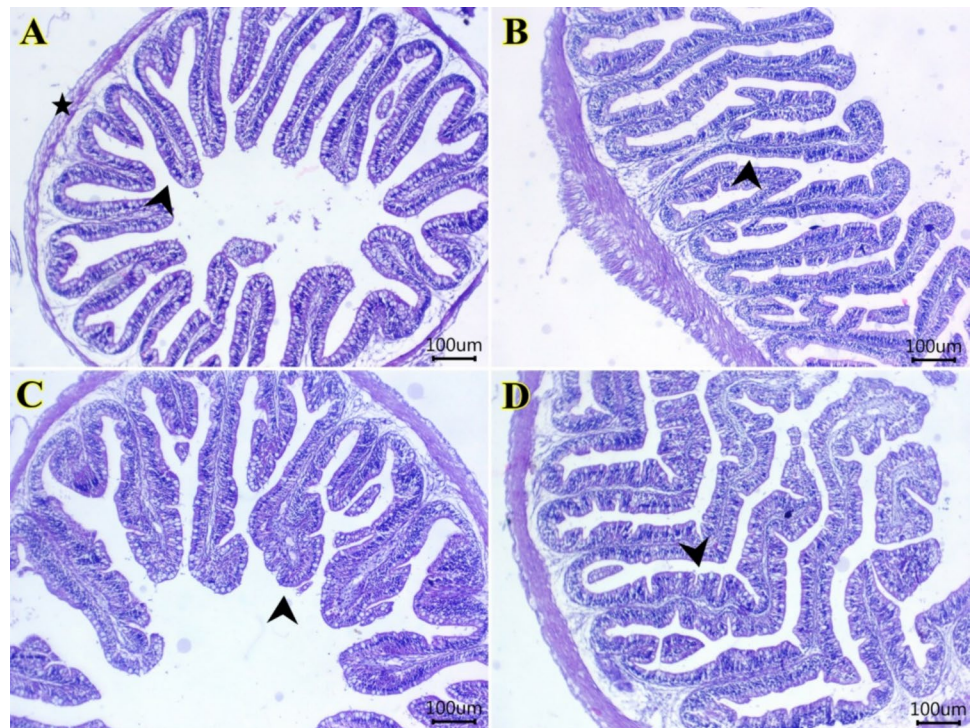
Discussion

In recent years algae have become valuable functional ingredients in aquafeeds, providing a sustainable source of bioactive compounds that promote fish growth, enhance feed utilization, and improve overall health (García-Vaquero and Hayes 2016; Naiel et al. 2021). This study found that incorporating *Halimeda* powder at a lower inclusion level (0.25%) significantly improved growth performance and feed conversion efficiency in Nile tilapia, while higher inclusion levels exerted negative effects, indicating a dose-dependent response.

The beneficial effects observed at lower inclusion levels are likely due to the presence of several bioactive compounds such as polysaccharides, flavonoids, terpenoids, and saponins, which are known to modulate gut health, stimulate digestive enzyme activity, and promote appetite (Francis et al. 2002; Yin et al. 2023; Khanzadeh et al. 2024a). In particular, saponins may enhance intestinal permeability and nutrient transport when provided in lower doses, while higher levels have been shown to compromise epithelial integrity and suppress digestive enzyme secretion, thus reducing nutrient utilization and growth (Francis et al. 2001, 2005).

These findings align with previous reports indicating that the effects of algae on aquaculture species depend greatly on dosage, species, and preparation methods. For instance, Khanzadeh et al. (2024b) demonstrated that a hydroalcoholic

Fig. 2 Photomicrograph of H&E stained sections from intestine samples ($n = 10$) showing: A (CNT): normal histological structures of mucosa (arrowhead), submucosa and muscular layer (star) in the control group. B (H0.25): maintain the integrity of mucosal layer with branched their villous surface (arrowhead) in the H0.25 fish group. Normal configurations of intestinal layers with broad most villous ends (arrowhead) in the H0.5 fish group. Increase villi height and corrugated surface area of their villous epithelium (arrowhead) in the H1 fish group. Scale bar = 100 μm



extract of *Laurencia caspica* improved growth and digestive enzyme activities in Nile tilapia. Similarly, dietary supplementation with 8.0 g kg^{-1} of *Padina pavonica* significantly improved growth performance under both experimental and farming conditions in Nile tilapia (Maghawri et al. 2023). Also, Shen et al. (2025) reported that dietary supplementation with 0.1% oligotriosaccharides and 0.2% brown algae powder enzymatic product significantly improved specific growth rate and reduced feed conversion ratio compared to the control group on largemouth bass. Likewise, fucoidan extracted from *Sargassum ilicifolium* was shown to enhance growth and antioxidant defensive mechanisms in Oscar fish (*Astronotus ocellatus*) (Khanzadeh et al. 2024b). In addition, many reports indicate similar biphasic effects of saponin-rich plant extracts in aquaculture, where low doses stimulated growth and higher doses had adverse effects (Hossain et al. 2004; Chen et al. 2011; Khanzadeh et al. 2025). Thus, the observed effects of *Halimeda* supplementation in this study are attributed to the biological activities of its saponin content in a dose-dependent manner.

In the same context, the enhanced digestive enzyme activities observed in fish fed the 0.25% *Halimeda* diet (H0.25) suggest improved feed digestion and nutrient assimilation, which likely contributed to the boost growth and feed utilization recorded at this inclusion level, without altering triglyceride concentrations. Consequently, histological analysis showed that fish in the H0.25 group displayed a preserved mucosal structure and increased villus branching at the intestinal tips, indicating a larger absorptive surface area

and improved nutrient uptake potential. These findings are consistent with previous reports showing that moderate levels of algae supplementation can enhance digestive enzyme secretion and gut morphology in various aquatic animals. For instance, Batista et al. (2020) demonstrated that dietary *Ulva* and *Gracilaria* improved digestive enzyme profiles and intestinal architecture in European seabass, while Abdelrhman et al. (2022) noted similar enhancements in hybrid red tilapia fed polysaccharides derived from the brown alga *Sargassum dentifolium*. The improvement in intestinal digestive function may be attributed to the role of polysaccharides derived from marine algae, which significantly influence the regulation of intestinal microbiota, thereby benefiting host health by stimulating the production of functional metabolites via intestinal microbiota (Jiang et al. 2022).

Evaluating serum protein constituents is essential for assessing fish health, as these parameters reflect the nutritional status, immune function, and overall physiological condition of aquatic organisms. In this study, fish fed the H0.25 diet exhibited significantly higher levels of serum protein constituents compared to the control group, demonstrating significant cubic trends. Additionally, hepatic enzyme activities, specifically aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were notably reduced in the H0.25 group, indicating improved liver function and decreased cellular damage. This supports the health-promoting effects of low-level *Halimeda* supplementation. These improvements are consistent with previous studies indicating that supplementation with microalgae boosts serum protein

levels while simultaneously reducing hepatic enzyme leakage, suggesting better metabolic efficiency and enhanced health in gilthead seabream (Perera et al. 2020) and *O. niloticus* (Ibrahim et al. 2022). Furthermore, Mansour et al. (2021) demonstrated that supplementing Nile tilapia diets with *Spirulina* at optimal levels (0.63–0.67%) significantly improved haemoglobin concentration, elevated plasma total protein and lipid levels, reduced glucose concentration, and decreased aminopeptidase activity, indicating enhanced metabolic efficiency and physiological status. Notably, the current study indicated significantly elevated levels of creatinine (CREA) in fish fed the 0.5% diet, representing that higher doses may induce mild metabolic or renal stress. On the other hand, it has been previously shown that *H. opuntia* is a vital source of bioactive compounds, including carotenoids, fats, proteins, dietary fibers, fatty acids, vitamins, and minerals (Nazarudin et al. 2022). Thus, the *Halimeda* supplementation might enhance protein metabolism, liver health, and immune function, likely due to its antioxidant carotenoids and higher omega-3 fatty acid content (Mandal et al. 2025).

Immune parameters such as lysozyme activity (LZM), cytokine expression (*IL-1 β* , *TNF- α*), and alternative complement activity (ACH50) serve as critical indicators of innate immunity in fish, representing the first defensive line against pathogens (Magnadóttir 2006; Saleh et al. 2024). In the present study, dietary supplementation with *H. opuntia* powder, particularly at 0.25%, significantly enhanced LZM and *IL-1 β* levels, while *TNF- α* levels were markedly reduced compared to the control group. These immunological improvements were further supported by upregulated hepatic expression of *PCNA* (proliferating cell nuclear antigen), which increased linearly ($P \leq 0.01$) with *Halimeda* inclusion, suggesting improved leukocyte proliferation and immune cell regeneration. The enhancement of immune responses can be attributed to the presence of various bioactive compounds in *Halimeda*, including sulphated polysaccharides, terpenoids, sterols, and polyphenols. Specifically, sulphated polysaccharides have been shown to activate immune-related signalling pathways, such as the Toll-like receptor (TLR) and NF- κ B pathways, thereby increasing the expression of cytokines like *IL-1 β* and improving lysozyme synthesis (Cadar et al. 2025). These compounds also act as immunostimulants by enhancing macrophage activity and complement system function, which explains the elevated ACH50 levels. Furthermore, the reduction in *TNF- α* levels suggests a balancing effect of *Halimeda* on pro- and anti-inflammatory signalling, likely mediated by sterol-mediated modulation of cyclooxygenase and lipoxygenase pathways, helping to prevent excessive inflammation and maintain immune homeostasis (Kraiem et al. 2024). Similar immunomodulatory effects have been reported in Nile tilapia fed *Ulva fasciata*, where lysozyme activity and anti-inflammatory cytokines were significantly

upregulated (Abo-Raya et al. 2024). Furthermore, dietary supplementation with 0.5–1% *Dictyota dichotoma* powder significantly enhanced total immunoglobulin and lysozyme activity in zebrafish, indicating improved humoral immunity and systemic immune status (Mahmoudi et al. 2022). Also, Safavi et al. (2019) demonstrated that *Ulva intestinalis* and *Gracilariopsis persica* extracts rich in sulphated polysaccharides enhanced immune markers in rainbow trout. In addition, a recent study demonstrated that brown algae extracts from *Sargassum horneri*, *Undaria pinnatifida*, and *Sargassum fusiforme* significantly enhanced macrophage proliferation, cytokine production (*IL-1 β* , *TNF- α* , *IL-6*), and phagocytic activity in murine macrophages, indicating their strong immunomodulatory potential (Yoon et al. 2024). Therefore, the observed immunostimulatory outcomes in this study likely stem from synergistic actions of *Halimeda*'s natural compounds in enhancing immune recognition, activating leukocyte proliferation, and modulating inflammation-related gene expression.

Oxidative stress biomarkers, such as antioxidant enzymes glutathione (GSH) and superoxide dismutase (SOD), along with malondialdehyde (MDA) as a lipid peroxidation product, are widely recognized as reliable indicators of physiological status and stress resilience in fish (Hoseinifar et al. 2020). In the present study, Nile tilapia fed 0.25% *H. opuntia* powder exhibited significantly elevated GSH and SOD activities and reduced MDA levels, suggesting a strong antioxidant status and enhanced cellular redox homeostasis. These biochemical findings were further supported by gene expression results, which showed a significant increase in hepatic SOD mRNA expression ($P \leq 0.01$), following a quadratic trend consistent with serum activity. However, other key antioxidant genes such as *CAT*, *GPx*, *GST*, and *GSR* were not significantly affected, indicating a selective regulatory effect at this supplementation level.

One of the central molecular mechanisms underlying this antioxidant response likely involves the *Nrf2* (nuclear factor erythroid 2–related factor 2) signalling pathway (Bian et al. 2025). *Nrf2* is a master transcription factor that regulates the expression of antioxidant enzymes by binding to antioxidant response elements (AREs) in the promoters of target genes, including *SOD*, *CAT*, *GPx*, and *GST* (Liu et al. 2021). Bioactive compounds present in *Halimeda*, particularly phenolic compounds, sulphated polysaccharides, terpenoids, and carotenoids like β -carotene, are known to activate the *Nrf2* pathway either by facilitating *Nrf2* nuclear translocation or by inhibiting its cytoplasmic repressor Keap1 (Kelch-like ECH-associated protein 1) (Begum et al. 2021). This activation stimulates the transcription of endogenous antioxidant enzymes, thereby strengthening the organism's defensive mechanisms against oxidative damage.

Similar findings have been reported in other algae-based studies. For example, Zhao et al. (2025) demonstrated that

Ulva prolifera supplementation enhanced GSH and SOD activities while reducing MDA levels in carp under oxidative stress. Likewise, Chen et al. (2021) exhibited that *Chlorella sorokiniana* improved redox balance in rainbow trout through *Nrf2*-mediated antioxidant enzyme upregulation. Given this context, the improved antioxidant profile observed in the present study can be mechanistically attributed to the activation of the *Nrf2*-ARE pathway by *Halimeda*-derived molecules, with SOD emerging as a particularly responsive target (Zhao et al. 2021). This effect is vital for maintaining cellular integrity and mitigating the detrimental effects of reactive oxygen species (ROS), ultimately supporting fish health and resilience under environmental or metabolic stress.

Conclusion

Supplementation with *H. opuntia* algae powder, particularly at 0.25%, significantly enhanced growth, feed efficiency, digestive enzyme activities, and immune responses in *O. niloticus*. Key improvements included elevated lysozyme activity, interleukin-1 β , glutathione (GSH), superoxide dismutase (SOD), and reduced malondialdehyde (MDA). Hepatic gene expression analysis confirmed upregulation of PCNA and SOD. Histological analysis showed enhanced intestinal villi structure, suggesting improved nutrient absorption. These results indicate that 0.25% *Halimeda* optimizes growth, immunity, and gut health in Nile tilapia. Future research should focus on evaluating *Halimeda* supplementation in other species, assessing meat quality, and dealing with the antinutritional factors present in the macroalgae.

Author contribution Mohammed A.E. Naiel and Nahla E.M. Ismael contributed to the conceptualization of the study. Eman M. Zaki, Doaa K. Khames, and Shima M. Elian were responsible for the methodology. Xiaolin Meng, Ahmed H. Arisha, Mohammed A.E. Naiel, and Shima M. Elian conducted formal analysis and investigation. Mohammed A.E. Naiel, Eman M. Zaki, and Doaa K. Khames prepared the original draft. Mohammed A.E. Naiel, Xiaolin Meng, and Ahmed H. Arisha contributed to writing—review and editing. Funding acquisition was carried out by Mohammed A.E. Naiel and Xiaolin Meng. Nahla E.M. Ismael and Xiaolin Meng provided resources. Supervision was performed by Mohammed A.E. Naiel. All authors have read and approved the final version of the manuscript.

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Data availability Available under reasonable request from the corresponding author.

Ethics approval The animal ethics guidelines were followed and approved by the Zagazig university animal ethics committee (ZUIACUC/2/F/89/2025).

Competing interest None

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