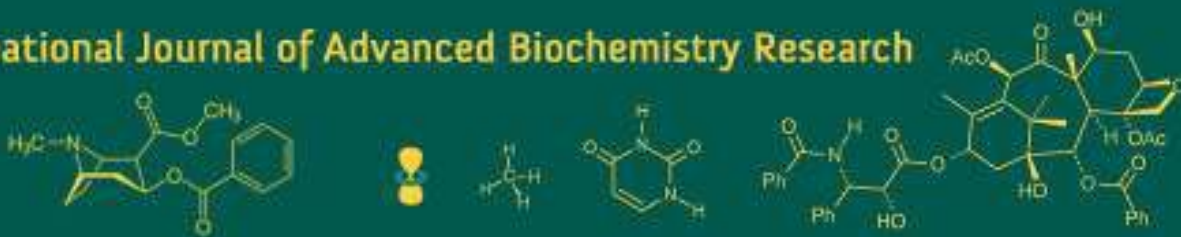


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Mahavadiya DR
 Assistant Professor,
 Department of Aquaculture,
 Faculty of Fisheries Science,
 The Neotia University,
 Kolkata, West Bengal, India

Joshi NH
 Associate Professor and Head,
 Centre of Excellence in
 Seaweed Research &
 Utilization, Kamdhenu
 University, Okha, Gujarat,
 India

Vishal Rathod
 Department of Aquaculture,
 Faculty of Fisheries Science,
 Veraval, Gujarat, India

Sapra Dharmesh
 Ph.D. Scholar, Department of
 Aquaculture, College of
 Fisheries Science, Kamdhenu
 University, Veraval, Gujarat,
 India

Corresponding Author:
Mahavadiya DR
 Assistant Professor,
 Department of Aquaculture,
 Faculty of Fisheries Science,
 The Neotia University,
 Kolkata, West Bengal, India

Physiological and metabolic responses of Nile tilapia (*Oreochromis niloticus*) fry to dietary substitution of fish meal with *Ulva* sp.

Mahavadiya DR, Joshi NH, Vishal Rathod and Sapra Dharmesh

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Abstract

This study investigated the physiological and metabolic responses of Nile tilapia (*Oreochromis niloticus*) fry to dietary substitution of fish meal with *Ulva* spp. over a 60-day feeding period. Fry were fed four isonitrogenous diets (40% crude protein) containing 0%, 5%, 10%, and 15% *Ulva* spp. Physiological assessments included antioxidant enzyme activities superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) along with hematological parameters such as hemoglobin concentration, red blood cell (RBC) count, white blood cell (WBC) count, and hematocrit value. Dietary inclusion of *Ulva* spp., particularly at 5%, significantly enhanced antioxidant enzyme activities and improved hematological indices compared to the control group ($P < 0.05$), indicating improved oxidative stress regulation and physiological health. Conversely, higher inclusion levels (10-15%) did not elicit further physiological benefits. The results suggest that moderate dietary inclusion of *Ulva* spp. effectively supports physiological homeostasis and health status in Nile tilapia fry, reinforcing its functional role in sustainable aquaculture nutrition.

Keywords: *Oreochromis niloticus*, *Ulva* spp., Antioxidant enzymes, Hematological parameters, Oxidative stress

Introduction

Aquaculture represents the fastest-expanding sector of global food production and has emerged as a critical component of global strategies aimed at ensuring food and nutritional security for a growing human population. The sector now contributes more than half of the aquatic food consumed worldwide and continues to expand at a rate surpassing that of capture fisheries and terrestrial animal production systems (FAO, 2020; FAO, 2022) [41, 18]. Among farmed freshwater species, Nile tilapia (*Oreochromis niloticus*) occupies a prominent position due to its rapid growth, efficient feed utilization, tolerance to a broad range of environmental conditions, omnivorous feeding habit, disease resilience, and high market acceptance. These favorable attributes have facilitated its widespread introduction and culture across diverse geographical regions and freshwater production systems, including ponds, reservoirs, rivers, irrigation canals, and recirculating aquaculture systems (El-Sayed, 2006; Lim & Webster, 2006; Anonymous, 2021) [12, 34, 2]. In 2022, global aquaculture production reached a historic peak, reflecting increasing consumer demand for affordable, high-quality animal protein and the growing reliance on aquaculture to bridge the gap between supply and demand for aquatic foods (FAO, 2022; OECD-FAO, 2023) [41, 18]. However, the intensification of aquaculture has led to increased dependence on formulated feeds, making feed cost one of the most significant constraints to economic sustainability. Feed expenses typically account for 30-70% of total production costs, depending on species, culture intensity, and ingredient availability (Rumsey, 1993; Tacon, 1997; Tacon & Metian, 2008) [48, 52, 53]. Consequently, the development of nutritionally balanced, cost-effective, and sustainable feed formulations remains a major research priority in tilapia aquaculture.

Fish meal (FM) has long been regarded as the benchmark protein ingredient in aquafeed formulations due to its high digestibility, balanced essential amino acid profile, superior palatability, and high bioavailability of micronutrients critical for fish growth and physiological functions (Hardy, 2010; Tacon & Metian, 2008) [25, 53]. However, the sustainability of FM utilization has been increasingly challenged by stagnation in global fish

meal production, declining wild fish stocks, escalating market prices, and growing environmental concerns associated with the overexploitation of marine ecosystems (Naylor *et al.*, 2009; Olsen & Hasan, 2012; FAO, 2020) [40, 43, 16]. The rapid expansion and intensification of aquaculture have further intensified pressure on finite marine resources and increased competition for FM with other animal production sectors, including poultry and livestock (Tacon & Metian, 2008; FAO, 2022) [53, 41]. Consequently, considerable research efforts have been directed toward identifying nutritionally adequate, environmentally sustainable, and economically viable alternative protein sources capable of partially or completely replacing FM in aquafeeds without adversely affecting growth performance, feed efficiency, or fish health (Gatlin *et al.*, 2007; Glencross *et al.*, 2012; Hua *et al.*, 2019; Mahmoud *et al.*, 2020) [20, 23, 29, 35]. In this context, a wide range of alternative feed ingredients, including plant-derived proteins, animal by-products, single-cell proteins, insect meals, and macro- and microalgae, have been extensively evaluated for their potential to reduce dependency on FM while supporting sustainable aquaculture development (Makkar *et al.*, 2014; Henry *et al.*, 2015; Glencross *et al.*, 2020) [37, 27, 23].

Macroalgae, commonly referred to as seaweeds, are broadly classified into green (Chlorophyta), red (Rhodophyta), and brown (Ochrophyta) groups, encompassing nearly 10,000 species worldwide, predominantly distributed in marine ecosystems (Kidgell *et al.*, 2019; Sullivan *et al.*) [31]. Seaweeds are increasingly recognized for their nutritional richness, providing valuable proteins, essential minerals, vitamins, and diverse bioactive compounds, thereby offering significant potential for both nutritional and functional applications in aquafeed formulations (Azenha).

Within green macroalgae, species of the genus *Ulva* have attracted increasing scientific and commercial interest as functional feed ingredients due to their relatively high protein content, favorable amino acid profile, richness in essential minerals, and suitability as partial substitutes for fish meal in aquaculture diets (Jamal *et al.*, 2017; Msuya & Neori, 2008; Fleurence *et al.*, 2012; Wan *et al.*, 2019) [30, 38, 19, 57]. In addition to their nutritional value, *Ulva* spp. are characterized by the presence of ulvan, a sulfated heteropolysaccharide that constitutes approximately 9-36% of the algal dry biomass and serves as the primary structural component of the cell wall (Usov, 2011; Lahaye & Robic, 2007; Kidgell *et al.*, 2019) [55, 33, 31]. Ulvan has been extensively reported to exhibit a broad spectrum of biological activities, including antioxidant, immunomodulatory, anti-inflammatory, anticoagulant, antiviral, and prebiotic effects, thereby conferring functional benefits beyond basic nutrition when incorporated into animal feeds (Hamed *et al.*, 2015; Pereira, 2018; Qi *et al.*, 2012; Wang *et al.*, 2020; Cunha & Grenha, 2016) [24, 46, 47, 58, 8]. Owing to their rapid growth rate, wide ecological tolerance, and year-round availability along tropical and subtropical coastlines, including the Gujarat coast of India, *Ulva* spp. represent a renewable and locally available biomass with strong potential for sustainable aquafeed development (Msuya *et al.*, 2014; Buschmann *et al.*, 2017; FAO, 2022) [39, 4, 41].

Among the species within this genus, *Ulva lactuca* (sea lettuce) has received particular attention due to its high concentration of bioactive compounds, including sulfated polysaccharides, phenolic compounds, essential amino

acids, vitamins, and trace elements, which collectively contribute to its antioxidant, antimicrobial, anti-inflammatory, and immunostimulatory properties (El-Baky *et al.*, 2008; Alghazeer *et al.*, 2013; Lahaye, 1991; Ortiz *et al.*, 2006; Putra *et al.*) [10, 1, 5, 55]. These attributes position *U. lactuca* as a promising functional additive and partial fish meal replacer in aquafeeds. Recent studies have demonstrated that dietary incorporation of *U. lactuca* enhances growth performance, feed utilization efficiency, digestive enzyme activity, antioxidant defense mechanisms, and immune responses in several cultured fish species, including Nile tilapia (*Oreochromis niloticus*) (Ergün *et al.*, 2009; Valente *et al.*, 2016; Dernekbaşı; Carvalho *et al.*, 2025) [13, 56, 5]. For example, Dernekbaşı reported significant improvements in growth performance, intestinal digestive enzyme activity, and antioxidant enzyme responses in Nile tilapia fed *U. lactuca*-supplemented diets under both fasting and normal feeding regimes. Similarly, Carvalho *et al.* (2025) [5] observed enhanced growth metrics and elevated antioxidant capacity in tilapia receiving graded dietary levels of *U. lactuca*, supporting its role as a functional feed ingredient in sustainable aquaculture nutrition.

Despite these promising outcomes, the optimal dietary inclusion levels of *Ulva* spp. and the underlying mechanisms responsible for their beneficial effects remain incompletely understood. Moreover, although positive responses have been documented in juvenile and adult fish, limited information is available regarding the effects of *Ulva* supplementation in fry, a developmental stage particularly sensitive to nutritional and environmental stressors. Investigating the role of *Ulva* spp. during early life stages is therefore essential to support healthy growth and physiological resilience in aquaculture systems.

This study assessed the effects of fish meal replacement with *Ulva* spp. on antioxidant enzyme activities and hematological indices in Nile tilapia fry, aiming to inform sustainable aquafeed development.

Materials and Methods

Experimental Fish Husbandry, Rearing, Feed, and Water Quality Monitoring

Healthy Nile tilapia (*Oreochromis niloticus*) fry were obtained from a commercial hatchery and transported carefully to the Inland Fisheries Research Station, Junagadh Agricultural University (JAU), Junagadh in oxygenated polythene bags to minimize transport stress. Upon arrival, the fry were acclimated for seven days in circular FRP tanks with continuous aeration and fed a standard control diet twice daily until they adjusted to laboratory conditions.

A total of 240 fry of similar size were randomly distributed into four dietary treatments, each with three replicates and 20 fry per replicate. The experimental diets included:

- **T₀ Control (FM):** 100% fish meal-based diet
- **T₁ (5 % *Ulva*):** 5% replacement of fish meal with *Ulva*
- **T₂ (10% *Ulva*):** 10% replacement of fish meal with *Ulva*
- **T₃ (15% *Ulva*):** 15% replacement of fish meal with *Ulva*

Fry were stocked at a density not exceeding 1 g/L, in accordance with standard laboratory practices. Feeding was conducted twice daily (10:00 and 16:00 h) to apparent satiation for the entire 60-day experimental period, and tanks were covered to prevent fish from jumping out.

Daily maintenance included manual cleaning and removal of uneaten feed and fecal matter by siphoning. An equal volume of fresh, aerated water was added after cleaning to maintain stable water levels. Environmental conditions such as temperature and light were left natural and were not deliberately altered during the study. Water quality parameters were closely monitored throughout the trial. Temperature and dissolved oxygen (DO) were recorded daily using a portable multiparameter meter, while pH was measured using a calibrated digital pH meter. Ammonia

levels were determined weekly using standard colorimetric test. Measurements were taken at consistent times each day to avoid disturbances, and any deviations from optimal ranges were corrected immediately through partial water replacement and adjustment of aeration. The water conditions were maintained within ranges suitable for tilapia fry: temperature 27-29 °C, dissolved oxygen 5.5-6.5 mg/L, pH 7.5-8.0, and ammonia <0.25 mg/L, providing a stable and stress-free environment throughout the experiment.

Table 1: Composition of experimental diet prepared using macroalgae (*Ulva* sp.)

	Diet prepared with 40 % protein			
	T ₀ (control)	T ₁ (5 % <i>Ulva</i>)	T ₂ (10 % <i>Ulva</i>)	T ₃ (15 % <i>Ulva</i>)
Fish meal	74	73	71	70
<i>Ulva</i>	0	4	8	12
Tapioca Powder	10	9	8	7
Wheat Flour	10	8	7	5
Plant oil	2	2	2	2
Fish oil	2	2	2	2
Vitamin and Mineral	2	2	2	2
Total	100	100	100	100

Sampling for Antioxidant Enzymes

For the assessment of antioxidant enzyme activities, the middle portion of the intestinal tissue was excised and homogenized in chilled 0.9% saline at a ratio of 1 g tissue to 9 mL solution, followed by centrifugation at 5000 × g for 15 minutes at 4 °C to remove debris. The resulting supernatant was collected under sterile conditions and stored at -20 °C until analysis.

Superoxide dismutase (SOD) activity was determined by combining 192.64 µL of Tris-EDTA buffer (pH 8.5), 6.7 µL of pyrogallol, and 0.66 µL of tissue homogenate, while the blank contained only buffer and pyrogallol, with absorbance measured at 420 nm using a UV-Vis spectrophotometer. Catalase (CAT) activity was assessed by adding 2 mL of dichromate/acetic acid reagent to the homogenate, which produced a blue color that turned green after incubation in a water bath at 37 °C for 10 minutes, and absorbance was recorded at 570 nm. GSH activity was evaluated by treating the homogenate with 5% sulfosalicylic acid, followed by centrifugation, and the yellow supernatant was read at 412 nm. Lipid peroxidation, indicated by malondialdehyde (MDA) levels, was measured by adding 600 µL of thiobarbituric acid (TBA) solution to 200 µL of sample or standard, incubating at 95 °C for 60 minutes, cooling for 10 minutes at room temperature, adding 300 µL of n-butanol, and measuring absorbance at 532 nm. These procedures collectively allowed a comprehensive evaluation of the antioxidant status and lipid peroxidation in Nile tilapia fry fed diets supplemented with *Ulva vulgaris*.

Hematological Parameters

At the end of the trial, hematological parameters including hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and hematocrit (HCT) were measured. Blood samples were collected by cardiac puncture using 1 mL syringes pre-rinsed with 2.7% EDTA. The puncture site was ventral, midway between the anterior bases of the pectoral fins. Collected blood was immediately transferred into Eppendorf tubes containing 20 µL of 2.7% EDTA and maintained on ice. Hematological measurements were performed using a fully automated blood cell counter, following standard protocols.

Statistical Analysis

All data, including growth performance, feed utilization, antioxidant enzyme activities, and hematological parameters, were analyzed using one-way analysis of variance (ANOVA) in SPSS software (version 26, IBM, Richmond, VA, USA). When significant differences were detected ($P < 0.05$), Tukey's HSD test was applied as a post-hoc comparison to identify differences between treatment means. All results are presented as mean ± standard error (SE).

Result

Antioxidant Enzymes

Dietary supplementation with *Ulva* significantly influenced the antioxidant enzyme activities in *O. niloticus* fry (Table 2). Superoxide dismutase (SOD) activity showed a dose-dependent increase with higher levels of *Ulva* inclusion. The control group (T₀) exhibited the lowest SOD activity (6.41 ± 0.30 U mg⁻¹ protein), which was significantly elevated ($p < 0.05$) in the 5% *Ulva* group (T₁, 7.10 ± 0.60 U mg⁻¹ protein), further increasing in the 10% (T₂, 8.30 ± 0.75 U mg⁻¹ protein) and peaking in the 15% *Ulva* group (T₃, 9.50 ± 0.80 U mg⁻¹ protein). This enhancement suggests improved dismutation of superoxide radicals in response to *Ulva* bioactive compounds.

In contrast, catalase (CAT) activity exhibited a significant inverse relationship with *Ulva* inclusion levels. The highest CAT activity was recorded in the control (5.18 ± 0.45 U mg⁻¹ protein), which decreased significantly in the 5% *Ulva* group (4.70 ± 0.44 U mg⁻¹ protein) and continued to decline progressively to the lowest activity observed in the 15% group (3.30 ± 0.50 U mg⁻¹ protein). The reduction in CAT activity might indicate a shift in the antioxidant defense mechanism or compensatory action by other enzymatic antioxidants such as GPX.

Glutathione peroxidase (GPX) activity increased significantly ($p < 0.05$) in all *Ulva*-supplemented groups compared to the control. The control group showed the lowest GPX activity (1.92 ± 0.18 U mg⁻¹ protein), whereas the 15% *Ulva* group recorded the highest activity (4.55 ± 0.40 U mg⁻¹ protein), with intermediate values in the 5%

(2.36 ± 0.28 U mg^{-1} protein) and 10% groups (3.20 ± 0.35 U mg^{-1} protein). The elevated GPX activity indicates enhanced detoxification of hydrogen peroxide and organic hydroperoxides, reflecting an upregulated enzymatic antioxidant defense in response to *Ulva* supplementation. Malondialdehyde (MDA) content, a biomarker of lipid peroxidation and oxidative stress, did not show significant differences ($p > 0.05$) across all treatment groups, remaining relatively stable between 1.24 ± 0.14 and 1.30 ± 0.18 nmol

mg^{-1} protein. This suggests that dietary *Ulva* inclusion at up to 15% did not induce oxidative damage in *O. niloticus* fry, despite modulation of enzymatic antioxidant activities. Collectively, these findings demonstrate that *Ulva* supplementation enhances specific components of the antioxidant defense system, particularly SOD and GPX activities, which may contribute to improved oxidative stress management and overall health status in Nile tilapia fry.

Table 2: Antioxidant enzyme activities of *O. niloticus* fry fed diets with different levels of *Ulva*. Values are mean $\pm$ SE (n = 3). Different superscript letters indicate significant differences ($p < 0.05$).

Parameter	T ₀	T ₁	T ₂	T ₃
SOD (U mg^{-1} protein)	6.41 ± 0.30^a	7.10 ± 0.60^b	8.30 ± 0.75^c	9.50 ± 0.80^d
CAT (U mg^{-1} protein)	5.18 ± 0.45^d	4.70 ± 0.44^c	4.08 ± 0.40^b	3.30 ± 0.50^a
GPX (U mg^{-1} protein)	1.92 ± 0.18^a	2.36 ± 0.28^b	3.20 ± 0.35^c	4.55 ± 0.40^d
MDA (nmol mg^{-1} protein)	1.26 ± 0.15^a	1.28 ± 0.16^a	1.24 ± 0.14^a	1.30 ± 0.18^a

Blood Indices

Dietary supplementation with *Ulva* at varying inclusion levels significantly affected several key hematological parameters in *Oreochromis niloticus* fry (Table 3). White blood cell (WBC) counts, an important indicator of immune competence and disease resistance, increased progressively and significantly ($p < 0.05$) with rising *Ulva* supplementation. The control group (T₀) exhibited a WBC count of $35.9 \pm 2.6 \times 10^3 \text{ mm}^{-3}$, which was comparable to the 5% *Ulva* group (T₁, $38.6 \pm 3.6 \times 10^3 \text{ mm}^{-3}$). However, significant increases were observed at the 10% (T₂, $44.0 \pm 4.4 \times 10^3 \text{ mm}^{-3}$) and 15% *Ulva* inclusion levels (T₃, $48.6 \pm 3.8 \times 10^3 \text{ mm}^{-3}$), suggesting an enhanced immunostimulatory effect of dietary *Ulva*, possibly attributable to its bioactive polysaccharides and antioxidants.

Red blood cell (RBC) counts showed a similar trend, with significant elevation ($p < 0.05$) from $1.62 \pm 0.16 \times 10^6 \text{ mm}^{-3}$ in the control group to $2.18 \pm 0.18 \times 10^6 \text{ mm}^{-3}$ in the highest *Ulva* supplementation group. The intermediate groups (T₁ and T₂) showed a stepwise increase, indicating improved erythropoiesis or maintenance of red cell populations, which likely enhances oxygen transport capacity and overall metabolic performance.

Hematocrit (HCT), representing the percentage of erythrocytes in blood volume, increased significantly and dose-dependently ($p < 0.05$) from $21.5 \pm 1.9\%$ in T₀ to $38.6 \pm 2.4\%$ in T₃. This rise in HCT is indicative of improved blood oxygen-carrying capacity and may correlate with

enhanced physiological condition and stress tolerance in fish fed higher *Ulva* levels.

Hemoglobin (Hb) concentration also increased significantly across dietary treatments, from $7.23 \pm 0.6 \text{ g dL}^{-1}$ in the control to $11.18 \pm 0.9 \text{ g dL}^{-1}$ in the 15% *Ulva* group. Elevated Hb levels complement the observed increases in RBC and HCT, collectively suggesting improved oxygen transport efficiency, which could support enhanced growth and metabolic activity.

Platelet counts showed a different pattern, with a significant increase ($p < 0.05$) in the 5% *Ulva* group ($415.2 \pm 35.0 \times 10^3 \text{ mm}^{-3}$) compared to control ($329.1 \pm 28.0 \times 10^3 \text{ mm}^{-3}$). However, platelet numbers declined in the 10% ($374.5 \pm 40.0 \times 10^3 \text{ mm}^{-3}$) and 15% groups ($332.0 \pm 37.0 \times 10^3 \text{ mm}^{-3}$), which were not significantly different from the control. This suggests that while moderate *Ulva* supplementation may stimulate thrombopoiesis or platelet activation, higher levels do not confer further benefits, potentially reflecting a homeostatic regulatory mechanism.

Overall, these hematological results indicate that dietary *Ulva* supplementation enhances the immune function and oxygen transport capabilities of Nile tilapia fry, particularly at moderate inclusion levels (5-10%), which may translate into improved health status and resilience under culture conditions. The increase in WBC, RBC, HCT, and Hb underscores *Ulva*'s potential as a functional feed additive, while the platelet response warrants further investigation to elucidate its role in fish hemostasis and immunity.

Table 3: Hematological parameters of *O. niloticus* fry fed diets with different levels of *Ulva*. Values are mean $\pm$ SE (n = 3). Different superscript letters indicate significant differences ($p < 0.05$).

Parameter	T ₀ (Control)	T ₁ (5% <i>Ulva</i>)	T ₂ (10% <i>Ulva</i>)	T ₃ (15% <i>Ulva</i>)
WBC ($\times 10^3 \text{ mm}^{-3}$)	35.9 ± 2.6^a	38.6 ± 3.6^a	44.0 ± 4.4^b	48.6 ± 3.8^c
RBC ($\times 10^6 \text{ mm}^{-3}$)	1.62 ± 0.16^a	1.87 ± 0.24^{ab}	2.04 ± 0.20^b	2.18 ± 0.18^b
HCT (%)	21.5 ± 1.9^a	27.8 ± 2.8^b	32.8 ± 3.2^c	38.6 ± 2.4^d
Hb (g dL^{-1})	7.23 ± 0.6^a	8.25 ± 0.9^b	9.27 ± 0.7^c	11.18 ± 0.9^d
Platelets ($\times 10^3 \text{ mm}^{-3}$)	329.1 ± 28.0^a	415.2 ± 35.0^b	374.5 ± 40.0^{ab}	332.0 ± 37.0^a

Throughout the feeding trial, no significant mortality occurred across any of the dietary treatments, suggesting that all diets were well tolerated by the *O. niloticus* fry. Key water quality parameters including temperature, pH, dissolved oxygen, and ammonia levels remained within the optimal ranges suitable for Nile tilapia culture and did not differ significantly between groups. This indicates that the observed variations in growth and physiological responses

were attributable primarily to the dietary inclusion of *Ulva*, rather than environmental fluctuations. Additionally, no signs of disease, stress, or abnormal behavior were observed in any treatment group, further confirming the safety and suitability of the experimental diets.

Overall, the data demonstrate that dietary inclusion of *Ulva* at moderate levels (5-10%) enhanced growth performance, feed utilization, antioxidant enzyme activities, and

hematological indices in *O. niloticus*, while higher supplementation (15%) did not provide additional benefits. These results provide a clear basis for further interpretation and discussion of the underlying mechanisms in the Discussion section.

Discussion

Antioxidant Enzyme Activity

The present study demonstrated that dietary supplementation with *Ulva* spp. significantly enhanced the antioxidant defense system in *Oreochromis niloticus* fry. Specifically, superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities increased in a dose-dependent manner with higher inclusion levels of *Ulva*, whereas catalase (CAT) activity declined slightly. Importantly, malondialdehyde (MDA) levels, a biomarker of lipid peroxidation and oxidative damage, remained unchanged across treatments. These findings indicate that *Ulva* supplementation strengthens the enzymatic antioxidant capacity of Nile tilapia without inducing oxidative stress, thus potentially improving fish health and resilience to environmental challenges.

SOD is a critical first-line enzymatic defense against reactive oxygen species (ROS), catalyzing the dismutation of superoxide radicals into hydrogen peroxide and oxygen, thereby preventing cellular oxidative damage (Siddik *et al.*, 2024) [51]. The observed increase in SOD activity suggests that bioactive compounds present in *Ulva*, such as sulfated polysaccharides and phenolic compounds, enhance the fish's ability to neutralize superoxide radicals effectively. Similarly, GPX, which reduces hydrogen peroxide and organic hydroperoxides, showed elevated activity correlating with *Ulva* inclusion level. This concurrent upregulation of SOD and GPX indicates a coordinated activation of the antioxidant defense network, likely driven by the synergistic effects of vitamins, minerals (notably selenium and zinc), and antioxidant phytochemicals abundant in *Ulva* biomass (Carvalho *et al.*, 2025; Sánchez-Machado, López-Cervantes, & López-Hernández, 2020) [5, 49].

In contrast, the decrease in CAT activity observed with increasing *Ulva* levels may reflect an adaptive enzymatic regulation where elevated GPX activity reduces the available substrate hydrogen peroxide, thereby lessening the need for catalase-mediated detoxification (Chakraborty, Chakrabarti, & Ghosh, 2021) [6]. This enzymatic shift aligns with prior research indicating that antioxidant enzymes operate synergistically to maintain oxidative homeostasis efficiently (Sánchez-Machado *et al.*, 2020) [49]. The stability of MDA levels across all dietary groups further confirms that lipid peroxidation was effectively controlled, demonstrating that dietary *Ulva* prevented oxidative damage to cell membranes even at higher inclusion levels.

These results align with recent evidence showing that dietary seaweed supplementation improves antioxidant defenses in various fish species. Siddik *et al.* (2024) [51] reported that *Ulva* extract enhanced antioxidant enzyme activities and immune responses in Nile tilapia, which corroborates the present findings. Moreover, comprehensive reviews highlight the role of macroalgal polysaccharides and phenolic compounds in modulating oxidative stress and enhancing fish health (Cheng, Liu, & Zhang, 2020; Sánchez-Machado *et al.*, 2020) [7, 49].

Mechanistically, *Ulva*'s antioxidant properties may stem from both direct free radical scavenging by bioactive

compounds and indirect stimulation of endogenous antioxidant enzyme gene expression (Chakraborty *et al.*, 2021) [6]. The abundant micronutrients in *Ulva*, particularly selenium and zinc, act as essential cofactors for SOD and GPX enzymes, facilitating their catalytic efficiency (Oliveira, Martins, & Silva, 2016) [42]. This multifaceted mode of action likely underpins the dose-dependent enhancements observed, with moderate supplementation (5-10%) yielding optimal antioxidant benefits. Higher inclusion levels may exhibit diminishing returns due to potential anti-nutritional factors or reduced digestibility inherent in excessive seaweed intake (Carvalho *et al.*, 2025) [5].

From an applied perspective, enhancing antioxidant capacity via natural feed additives such as *Ulva* offers significant advantages in aquaculture, including improved stress tolerance, disease resistance, and growth performance (Siddik *et al.*, 2024) [51]. Oxidative stress is a key limiting factor in intensive aquaculture systems, and dietary strategies that bolster antioxidative defenses contribute to healthier and more productive fish stocks. Thus, the observed upregulation of SOD and GPX in *Ulva*-fed Nile tilapia highlights the potential of incorporating macroalgae as a functional feed ingredient to promote sustainable aquaculture practices.

In conclusion, this study provides compelling evidence that dietary *Ulva* supplementation supports the antioxidant defense system of *O. niloticus* fry, with moderate inclusion levels (5-10%) delivering the most pronounced benefits. The maintenance of stable MDA levels across treatments confirms effective management of oxidative stress, endorsing *Ulva* as a promising natural antioxidant source for aquafeeds.

Hematological Parameters

The hematological indices assessed in this study reveal that dietary supplementation with *Ulva* spp. significantly modulates the physiological and immune status of *Oreochromis niloticus* fry. Key parameters, including white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), hemoglobin (Hb), and platelet counts, exhibited marked changes correlated with the level of *Ulva* inclusion. These findings collectively indicate improved health status, especially at moderate supplementation levels.

WBC counts, an essential indicator of the innate immune response, increased significantly with *Ulva* supplementation, peaking in the highest inclusion group (T₃). Elevated leukocyte levels are indicative of enhanced immunocompetence, as WBCs are vital for pathogen recognition and clearance as well as for maintaining physiological homeostasis (Shang *et al.*, 2019) [50]. The immunostimulatory effect of *Ulva* may be attributed to its rich content of sulfated polysaccharides and phenolic compounds, which have been demonstrated to promote leukocyte proliferation and activate immune signaling pathways in fish (Wang *et al.*, 2020; He *et al.*, 2019) [59, 26].

Similarly, the observed increases in RBC counts, hemoglobin concentration, and hematocrit percentage suggest an improved oxygen transport capacity and metabolic efficiency in *Ulva*-fed fish. These parameters are directly related to erythropoiesis and blood oxygen-carrying potential, and their enhancement supports the notion that dietary *Ulva* can facilitate better physiological function and growth (El Gamal, 2010; Giri *et al.*, 2019) [9, 21]. Hematocrit increases parallel RBC and Hb improvements, reflecting

enhanced circulatory system performance and possibly better adaptation to metabolic demands.

Platelet counts displayed a unique pattern, with the highest counts observed in moderate inclusion (T_1) but declining at higher levels. Platelets play crucial roles not only in hemostasis but also in immune responses and tissue repair in fish, suggesting that optimal *Ulva* inclusion supports these physiological functions (Osman *et al.*, 2021) [45]. The decline in platelet counts at the highest inclusion level may be linked to anti-nutritional factors or decreased palatability affecting feed intake, as reported in some seaweed supplementation studies (Makkar *et al.*, 2016) [36].

The hematological improvements noted in this study are consistent with prior research demonstrating that dietary seaweed supplementation positively influences blood parameters in aquaculture species. For example, El-Sayed and Teshima (1992) [11] reported enhanced hematological and immunological parameters in tilapia fed seaweed extracts. More recently, Wang *et al.* (2020) [59] documented increased WBC and RBC counts in grass carp (*Ctenopharyngodon idella*) following dietary supplementation with *Ulva* polysaccharides. These findings corroborate the potential of seaweed-derived bioactive compounds to improve fish health by modulating

hematopoietic and immune functions.

The beneficial effects on hematological indices likely stem from the rich nutritional profile of *Ulva*, which contains essential vitamins (e.g., B-complex, C), minerals such as iron and zinc, and bioactive molecules including polysaccharides and phenolics. Iron is a critical component of hemoglobin and necessary for effective erythropoiesis, while vitamins and minerals contribute to immune cell proliferation and function (FAO, 2018; Becker, 2007) [14, 3]. Moreover, *Ulva*'s bioactive compounds may stimulate hematopoietic growth factors and cytokines, thereby enhancing both red and white blood cell production (Holdt & Kraan, 2011) [28].

Together with the observed enhancements in growth and antioxidant enzyme activities, the hematological improvements highlight that moderate dietary *Ulva* inclusion (5-10%) confers comprehensive benefits to the physiological and immunological status of *O. niloticus*. This is especially valuable under intensive aquaculture conditions, where fish encounter multiple stressors such as high stocking densities, water quality fluctuations, and pathogen pressures. The incorporation of *Ulva* as a functional feed additive thus holds promise for improving fish robustness and sustainable aquaculture productivity.

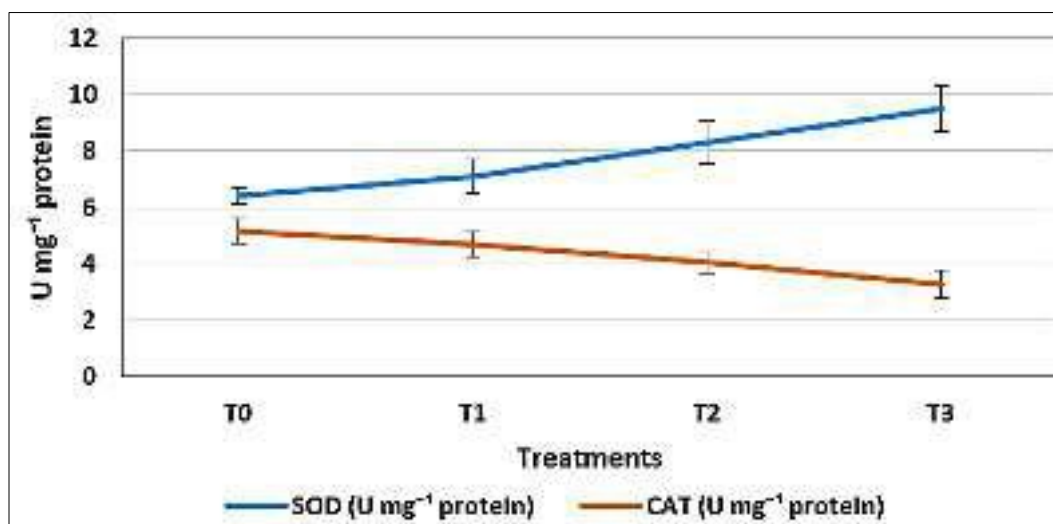


Fig 1: SOD and CAT enzyme activity of *O. niloticus* fry fed diets with different levels of *Ulva*

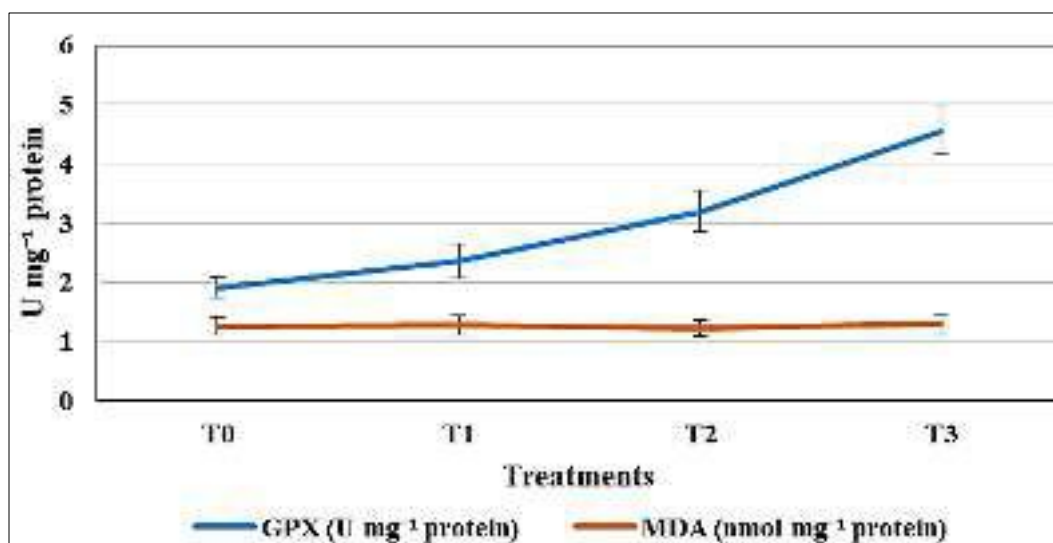


Fig 2: GPX and MDA enzyme activity of *O. niloticus* fry fed diets with different levels of *Ulva*

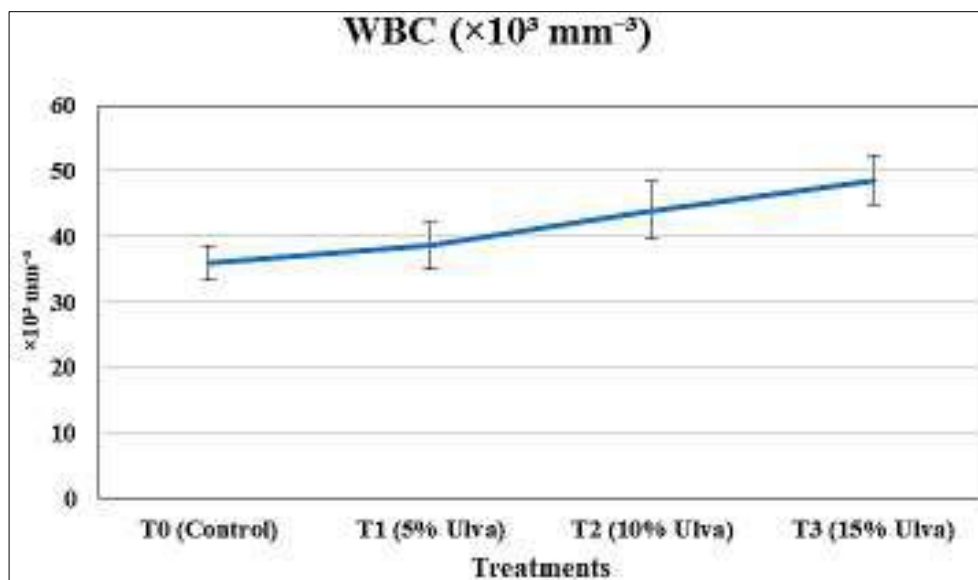


Fig 3: WBC count of *O. niloticus* fry fed diets with different levels of *Ulva*

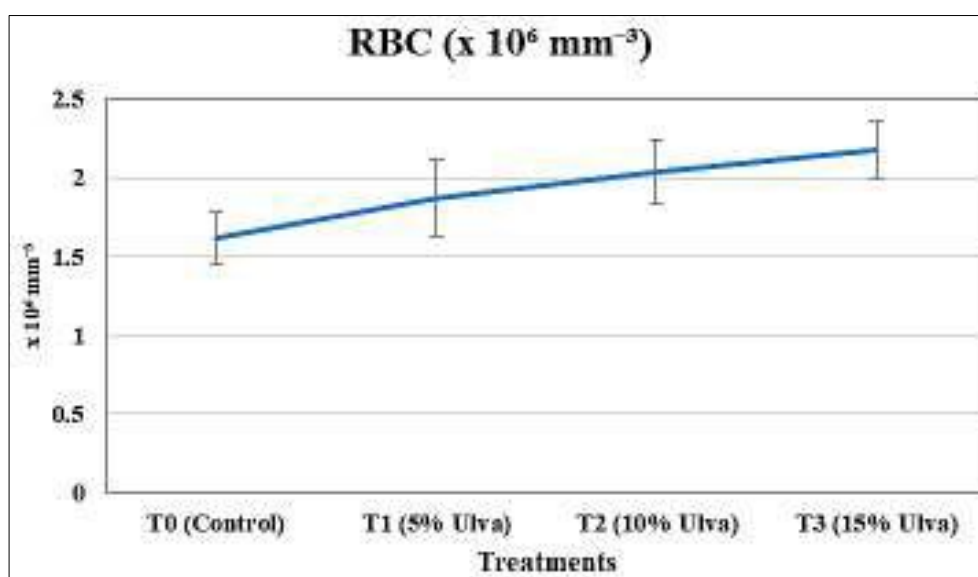


Fig 4: RBC count of *O. niloticus* fry fed diets with different levels of *Ulva*

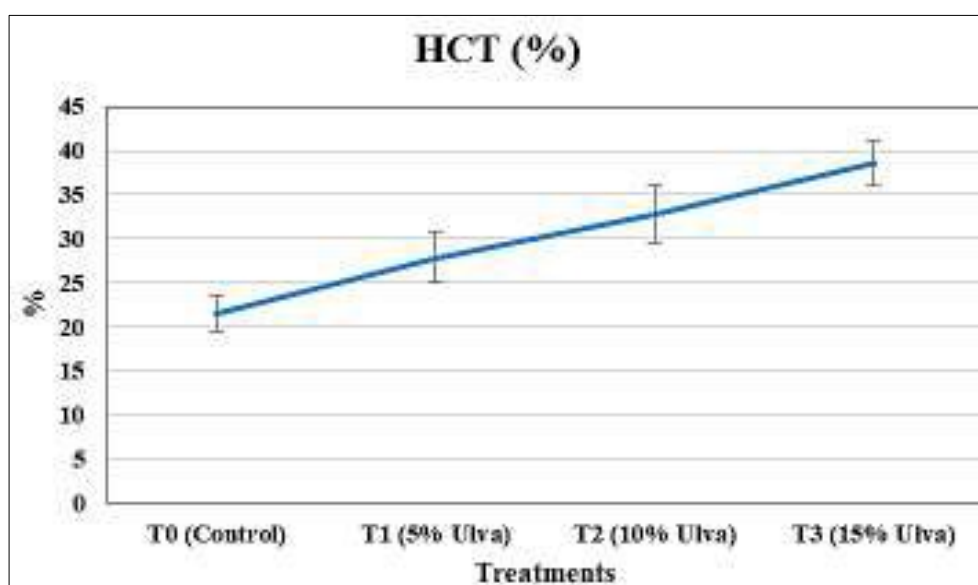


Fig 5: Hematocrit (%) of *O. niloticus* fry fed diets with different levels of *Ulva*

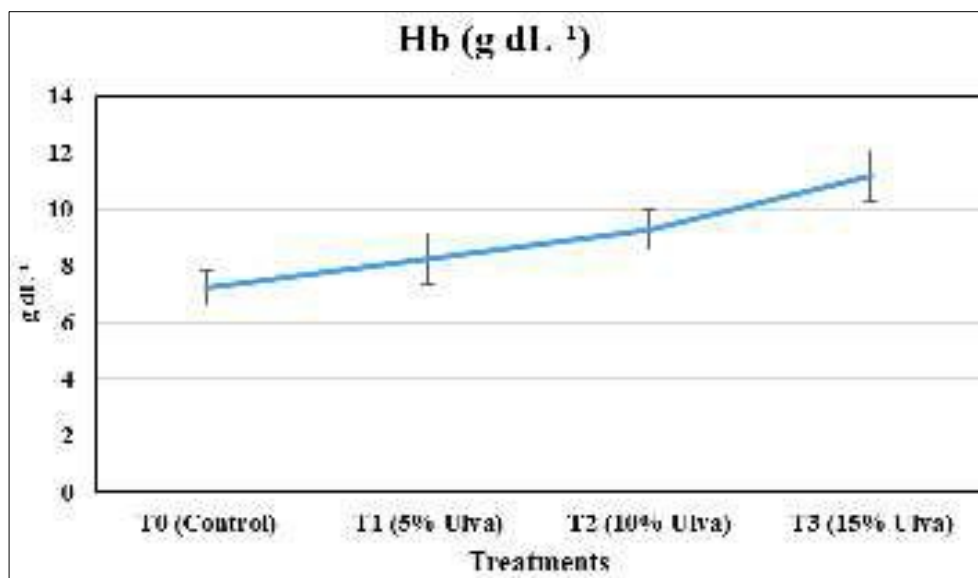


Fig 6: Hemoglobin levels of *O. niloticus* fry fed diets with different levels of *Ulva*

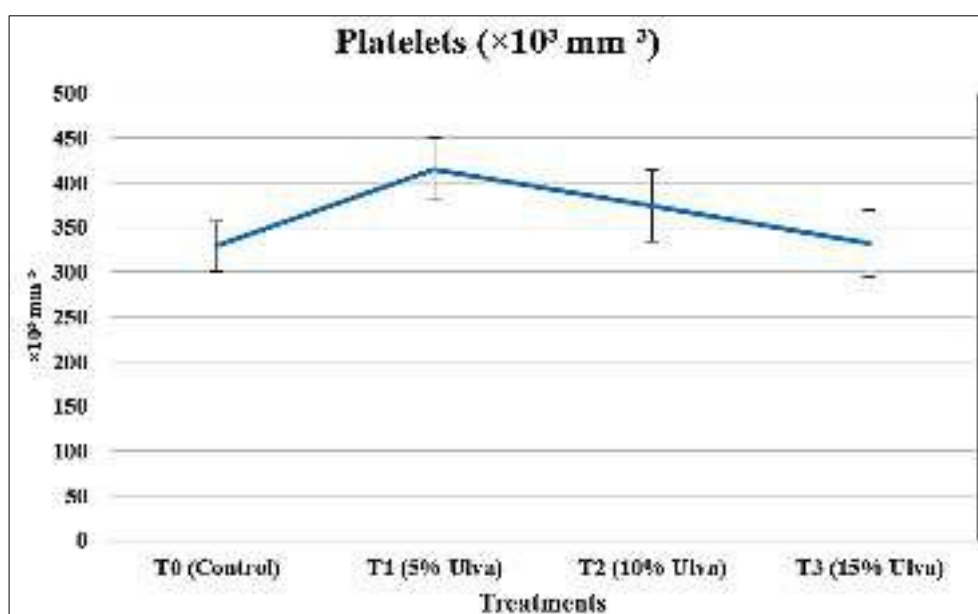


Fig 7: Platelet count of *O. niloticus* fry fed diets with different levels of *Ulva*

Conclusion

Overall, these findings suggest that 5-10% *Ulva* inclusion optimizes hematological parameters, supporting growth, immunity, and overall fish health. Higher levels of supplementation (15%) do not offer additional benefits and may slightly reduce performance, emphasizing the importance of determining optimal inclusion rates for functional feed ingredients.

Ulva supplementation positively influenced antioxidant activity and hematological parameters. Activities of key antioxidant enzymes, particularly superoxide dismutase and glutathione peroxidase, increased with *Ulva* inclusion, while catalase activity showed a slight decline and malondialdehyde levels remained stable, indicating enhanced oxidative defense and cellular stability. Furthermore, hematological indices such as white blood cells, red blood cells, hemoglobin, hematocrit, and platelet counts were elevated at moderate inclusion levels, reflecting improved immune response, oxygen-carrying capacity, and overall physiological condition. These beneficial effects are likely associated with the nutrient-rich composition and

bioactive compounds present in *Ulva*, including polysaccharides, vitamins, and trace minerals, which collectively support antioxidant defense and hematopoiesis. Overall, a 5% dietary inclusion of *Ulva* appears optimal for improving health and performance in tilapia aquaculture in a sustainable manner.

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