

Research Article

Effects of Dietary Phytol Supplementation on Growth Performance, Immunological Parameters, Intestinal Bacteria, and Prevention of Oxidative Stress Following Transportation of Nile Tilapia, Oreochromis niloticus

Saman Ahani⁽¹⁾,¹ Sara Ahani⁽¹⁾,¹ Morteza Yousefi⁽¹⁾,² Ali Taheri Mirghaed⁽¹⁾,³ and Afaf N. Abdel Rahman⁽¹⁾,⁴

¹Science and Research, Islamic Azad University, Tehran, Iran

²Department of Veterinary Medicine, RUDN University, Miklukho-Maklaya St, Moscow 117198, Russia ³Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran ⁴Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, P.O. Box 44511, Zagazig, Egypt

Correspondence should be addressed to Ali Taheri Mirghaed; mirghaed@ut.ac.ir

Received 30 September 2023; Revised 10 January 2024; Accepted 25 January 2024; Published 7 February 2024

Academic Editor: Mansour Torfi Mozanzadeh

Copyright © 2024 Saman Ahani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Nile tilapia, *Oreochromis niloticus*, $(2.00 \pm 0.02 \text{ g})$ were reared in 16 70-L tanks (40 individual/tank) and fed diets (approx. 345 g/kg protein, approx. 87 g/kg crude fat) containing 0 (CTL), 75 (PH-75), 150 (PH-150), and 300 (PH-300) mg/kg phytol (n = 4). After 60 days of feeding (4% daily), growth performance, humoral immune parameters, and gut bacteria were analyzed. Also, hepatic antioxidant parameters were determined before and after the fish were transported in plastic bags for 6 hr. The results showed that PH-75 exhibited the highest final weight (P < 0.001), weight gain (P < 0.001), feed intake (P < 0.001), feed efficiency (P = 0.015), plasma lysozyme activity (P = 0.004), and intestinal *Lactobacillus* sp. population (P = 0.017), among the treatments. The highest plasma alternative complement activity (P = 0.006) and the lowest intestinal total viable bacteria (P = 0.027) were observed in PH-75 and PH-150. The highest plasma alkaline phosphatase activities were observed in PH-75 and PH-300 (P = 0.014). The highest blood leukocyte (P = 0.008), monocyte (P = 0.001), and eosinophil (P < 0.001) were observed in PH-300, while the highest blood neutrophil was observed in all phytol treatments (P < 0.001). The highest hepatic lipid peroxidation was observed in PH-75, also PH-150 showed the lowest values (P < 0.001). The highest hepatic reduced glutathione was observed in PH-75, also PH-150 exhibited significant elevation in this parameter, compared to CTL (P < 0.001). Transportation led to significant elevations in the hepatic antioxidant enzymes' activities in CTL, PH-75, and PH-150; the highest activities were related to PH-75 and PH-150 treatments, which had also the highest post-transportation survivals (P < 0.001). In conclusion, phytol is a suitable feed supplement for Nile tilapia, improving growth performance and welfare, particularly at 75 mg/kg.

1. Introduction

The Nile tilapia, *Oreochromis niloticus*, which has a global production of 4.44 million tons in 2019, is the most widely cultivated tilapia species [1]. Nevertheless, the industry of Nile tilapia aquaculture is vulnerable to disease outbreaks, making it imperative to enhance fish health and prevent such occurrences [2]. Furthermore, global regulatory bodies have prohibited the use of chemical drugs and antibiotics in

aquaculture [3]; thereby, obliging fish farmers to focus on enhancing fish health and preventing disease outbreaks by using functional diets, among other managerial strategies [4].

Studies have demonstrated that herbal feed additives have various beneficial properties, including promoting growth, stimulating the immune system, acting as antioxidants, and tackling microbial infections. It has been found that these additives improve the overall health and immune responses of host organisms. They have also been shown to be effective

Ingredients (g/kg)	Dietary phytol levels (mg/kg)			
	0 (CTL)	75 (PH-75)	150 (PH-150)	300 (PH-300)
Corn meal ^a	123	123	123	123
Wheat flour ^b	241	241	241	241
Soybean oilcake ^c	306	306	306	306
Poultry by-product ^d	252	252	252	252
Fish canning by-product ^e	30	30	30	30
Plant oil (corn oil + sunflower oil; 1:1 ratio)	21	21	21	21
Vitamin + mineral premix ^f	15.5	15.5	15.5	15.5
Methionine ^g	3	3	3	3
Lysine ^h	3	3	3	3
Salt	5	5	5	5
Bentonite ⁱ	0.5	0.425	0.35	0.2
Phytol ^j	0	0.075	0.15	0.3
Proximate composition (g/kg)				
Moisture	88.6	88.3	88.3	88.7
Crude protein	345	348	341	344
Crude fat	87.5	86.4	88.1	86.7
Crude ash	69.1	69.0	68.2	68.0
Crude fiber	37.1	37.1	38.0	36.8

TABLE 1: Dietary ingredients and proximate composition.

^aContaining 8.9%, 3.5%, 5.3%, and 2.6% of crude protein, fat, ash, and fiber, respectively. ^bContaining 11.1%, 1.5%, 2.3%, and 2.5% of crude protein, fat, ash, and fiber, respectively. ^cContaining 44.3%, 1.88%, 5.32%, and 3.68% of crudeprotein, fat, ash, and fiber, respectively. ^dContaining 63%, 16%, 10%, and 4% of crude protein, fat, ash, and fiber, respectively. ^eContaining 56%, 18%, 4%, and 2% of crude protein, fat, ash, and fiber, respectively. ^fSupplied by Amineh Gostar Co., Tehran, Iran, providing per kg if diet: B2: 10 mg; E: 20 mg; K: 24 mg; B3: 12 mg; B5: 40 mg; B6: 5 mg; B1: 4 mg; A: 1,600 IU; D3: 500 IU; H: 0.2 mg; B9: 2 mg; B12: 0.01 mg; C: 60 mg; Inositol: 50 mg; Iodate: 0.05 mg; Fe: 2.5 mg; Co: 0.04 mg; Cu: 0.5 mg; Zn: 6 mg; Choline: 150 mg; Se: 0.15 mg; Mn: 5 mg. ^gCJ CheilJedang Corporation, Seoul, South Korea. ^hDomisha Industrial Production Co., Tehran, Iran. ⁱNinety nine percent purity; Sigma–Aldrich Co., Saint Louise, USA.

in stress management in aquaculture [5–7]. Stress is a common problem in farmed fish, which can disrupt their physiological balance and affect their growth, immune system, and health of various organs [8]. The transportation of fish, a common practice in aquaculture, can exacerbate these stress-related problems and lead to oxidative stress and immunosuppression [9–11]. However, studies have shown that certain herbal supplements, such as turmeric, *Curcuma longa* [10], and aloe vera, *Aloe vera* [12], can mitigate these negative effects. Despite this, there is still a need for further research on new herbal additives that can mitigate the disadvantages of transportation in fish.

The phenolic compounds found in plants have beneficial properties that make them suitable for inclusion in fish feed. Anti-inflammatory, antimicrobial, and antioxidant activities of these compounds can mitigate the negative effects of stress [13]. Synthetic forms of these compounds, known as nature identical compounds (NICs), offer advantages over natural compounds. Since NICs are more readily available and more cost-effective to produce, they are a practical choice for the aquaculture industry [13].

Phytol is a NIC with immune boosting, antibacterial, and antiviral properties. The production of phytol is widespread, as it serves as a substrate for the production of vitamins E and K [14]. The benefits of phytol in fish have only been examined in a few studies. For example, goldfish, *Carassius auratus*, fed with a feed containing phytol showed resistance to bacterial infection and improvement in hematological parameters after infection [15]. The antioxidant, antistress, and hepatoprotective properties of phytol helped mitigate the toxic effects of water ammonia on common carp, *Cyprinus carpio*, by acting as a hepatoprotective agent [16]. These results suggest that phytol may be a promising feed additive in aquaculture, but further investigation is needed to understand its physiological functions in other fish species.

Therefore, this study sought to evaluate the effects of dietary phytol incorporation on the growth performance, immunological parameters, post-transportation survival, and antioxidant responses of Nile tilapia.

2. Materials and Methods

2.1. Diets. Diets of similar macronutrient compositions were formulated using Windows User-Friendly Feed Formulation (WUFFDA-v.1). The only difference in diet was the amount of phytol added at the expense of bentonite, and four levels of phytol (0 (CTL), 75 (PH-75), 150 (PH-150), and 300 (PH-300) mg/kg) were tested (Table 1). Before addition to the other ingredients, phytol was mixed with the dietary oils. The ingredients were mixed for 20 min and moisturized to form a dough, which was then processed into pellets using a meat grinder. The pellets were dried overnight and stored at 4° C until use. The proximate compositions of the diets were determined according to AOAC [17]. The crude protein was determined using the Kjeldahl method after it was digested in sulfuric acid and the resulting ammonia was bound with boric acid. The solution was then titrated and the amount of nitrogen was determined and multiplied by 6.25 to calculate the crude protein content. Crude fat was determined as an ether extract using petroleum ether as a solvent in a Soxhlet apparatus (Gerhardt, Germany). Crude ash was measured using an electric furnace (550°C; 8 hr) by burning a 1-g sample and subtracting the weight of the sample before and after burning. Crude fiber was measured by placing a 1-g sample in a filter bag, boiling in 0.1 M NaOH, and then boiling in 0.1 M HCl. The crude fiber content was calculated by subtracting the weights of the samples before and after digestion. The moisture content of the samples was determined in the oven (105°C; 24 hr) by subtracting the weights of the samples before and after burning the samples before and after digestion.

2.2. Feeding Trial. Eight hundred Nile tilapia (~1.5 g) were purchased from a local farm (Kashan, Iran) and brought to the laboratory where they were housed in a 1,000-L tank. The fish were acclimatized to the conditions for 1 week by feeding them with CTL diet. They were then sorted to exclude individuals with outlier sizes (10% of the highest and lowest sizes). The remaining 640 fish were housed in 16 70-L tanks (58 cm \times 30 cm \times 40 cm) with 40 fish per tank. The fish within each tank were bulk-weighed both prior to stocking (total biomass of 77.2–83.2 g/per tank) and biweekly after that (to adjust the feed amounts). The fish were daily fed equal to 4% of biomass, divided into three meals (1.4% at 7:00, 1.4% at 13:00, and 1.2% at 18:00). No feed wastes were detected 20 min after each meal.

The tanks were thoroughly cleaned on a weekly basis and regularly aerated. The water quality was monitored using digital instruments, including a temperature of 27.3 ± 0.73 °C, dissolved oxygen of 6.60 ± 0.76 mg/L, pH of 7.4 ± 0.53 , and total ammonia of 0.85 ± 0.09 mg/L. The photoperiod was adjusted to a 12-hr light/dark cycle by means of lamps, and the fish were reared in these conditions for a duration of 60 days.

2.3. Growth Parameters. The growth and feed efficiency parameters were determined in all treatments according to the following equations:

Weight gain (%) =
$$100 \times \left[\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}\right],$$
(1)

Feed efficiency (%) =
$$100 \times \left[\frac{\text{Final weight} - \text{Initial weight}}{\text{Feed intake}}\right]$$
, (2)

Specific growth rate (SGR; %/day)
=100 ×
$$\left[\frac{\ln_{\text{Finalweight}} - \ln_{\text{Initialweight}}}{60}\right]$$
. (3)

2.4. Pretransportation Sampling and Processing. After 60 days, three fish per tank were gently caught by a dip net, anesthetized in a eugenol bath (50 μ L/L), and their blood

samples were taken for counting the blood leukocytes (WBC) and assessing plasma immunological parameters. The blood samples were collected by caudal puncture using heparinized syringes and collected in 2-mL plastic tubes. A portion of the blood samples were used for hematological assay and the other portion was centrifuged (4°C for 7 min; 5,000x g) for plasma separation, which was kept at -70° C until analysis. The fish were then euthanized by a sharp blow to the head, and their weights were recorded. To assay antioxidant parameters, the fish abdominal cavities were opened and a piece of the liver was dissected, washed with distilled water, frozen in liquid nitrogen, and stored at -70°C until analysis. A piece of the posterior intestine was then dissected, washed with sterile physiological saline, and immediately used for microbial culture. One additional batch of three fish per tank was caught, anesthetized, and then used for indirect skin mucus collection. The fish were individually placed in separate plastic bags containing 5 mL of Trisbuffered saline, comprising 50 mM Tris-HCl, 150 mM NaCl, pH 8.0 [18], and subsequently rubbing for a brief period. The combination of the mucus and buffer was collected and centrifuged at 4°C for a duration of 15 min (13,000 xg). The supernatants were collected and stored at a temperature of -70° C until the immunological analysis.

2.5. Analysis

2.5.1. Hematological Assays. Fresh blood samples were used for WBC counting, using the Dacie diluting solution according to Dacie and Lewis [19]. Differential WBC count was performed after preparation of the blood smear and staining by Giemsa, following Blaxhall [20].

2.5.2. Plasma Immunological Assays. The evaluation of plasma lysozyme activity was conducted by analyzing the lysis rate of *Micrococcus luteus*, as per Ellis [21]. A phosphate buffer pH 6.2 was used as the reaction medium. Thirty microliters plasma was mixed with 1 mL of the bacterial suspension and decreases in optical density was recorded for 5 min at 550 nm. Each 0.001 decrease in the optical density per minute was deemed as one unit of lysozyme activity. The plasma alkaline phosphatase (ALP) activity was determined using a commercial kit (Man Co., Tehran, Iran) as suggested by Esmaeili et al. [22]. This kit works by hydrolysis of p-nitrophenylphosphate (substrate) and production of p-nitrophenol with a yellow coloration. During 3 min, the optical density was measured at 405 nm and the enzyme's activity was calculated using a standard solution furnished by the manufacturer.

The activity of plasma alternative complement (ACH50) was determined by measuring hemolytic activity against sheep erythrocytes. The reaction medium was a barbital buffer (pH 7.0) containing ethylene glycol-bis(-aminoethyl ether)-N, N,N', N'-tetraacetic acid, magnesium, and gelatin. A defibrinated blood of sheep, obtained from Darvash Co., Tehran, Iran, underwent a three-step process of washing with the barbital buffer, followed by the suspension of $50 \,\mu$ L of the packed cells in 950 μ L of the sheep erythrocyte

suspension, $50 \,\mu\text{L}$ of the samples, and $50 \,\mu\text{L}$ of the buffer were added and mixed gently. After 90 min of incubation at room temperature, the hemolytic activity was stopped by adding a barbital buffer containing ethylenediaminetetraacetic acid. After a period of 5 min of centrifugation, the hemolysis of the tubes was determined at 420 nm, and the amount of sample producing 50% hemolysis was quantified through a log–log plot. The ACH50 activity was calculated according to Yano [23].

2.5.3. Skin Mucosal Immunological Assays. The skin mucosal lysozyme and ALP activities were determined as described for the plasma samples. The skin mucosal peroxidase activity was determined using 3,3', 5,5'-tetramethylbenzidine hydrochloride (TMB), as the substrate, and hydrogen peroxide. The dissolved TMB was dissolved in dimethylsulfoxide to obtain a concentration of 41 mM. To prepare a working solution of TMB, $200 \,\mu\text{L}$ of this solution was added to 8 mL of acetate buffer (205 mM, pH 4.0). Afterward, $500 \,\mu$ L of the TMB working solution, $250 \,\mu\text{L}$ of the samples, and 340 μ L of hydrogen peroxide (30%) were mixed and left at 40°C for 10 min. Subsequently, 1.2 mL of sulfuric acid 0.2 M was incorporated into the mixture to halt the reaction. After centrifugation (9000x g for 4 min), the absorbance of the supernatant was measured at 450 nm. The absorbance of the samples at the time zero was obtained by incorporating sulfuric acid at the time zero. The photooxidation of TMB was estimated using a control sample [24].

The soluble protein concentrations of the mucus homogenates were measured using the pyrogallol red method, using a commercial kit from Zist Chem Co., Tehran, Iran.

2.5.4. Hepatic Antioxidant Parameters' Assays. The determination of hepatic antioxidant parameters was conducted using commercial kits supplied by Zellbio Co. (Deutschland, Germany). The concentration of glutathione (GSH) was determined by measuring its reaction with 5,5 -dithiobis-(2nitrobenzoic acid) at 412 nm. The glutathione peroxidase (GPx) assay involved the addition of GSH to the samples and subsequent conversion into oxidized glutathione (GSSG) by the GPx enzyme present in the samples. It is then converted back to GSH by glutathione reductase activity, which requires nicotinamide adenine dinucleotide phosphate (NADPH). The decrease in NADPH concentration is directly proportional to the activity of GPx, as measured at 340 nm. The identical procedure is followed for determining GR activity, with the exception of incorporating GSSG into the samples in lieu of GSH. The content of thiobarbituric acid reactive substances (TBARS) was determined based on the reaction with thiobarbituric acid at 95°C, after deproteinization with trichloroacetic acid and in the presence of butylated hydroxytoluene. The pink coloration intensity was proportional to the TBARS concentration and was measured at 550 nm.

The soluble protein concentrations of the liver homogenates were measured using the pyrogallol red method, using a commercial kit from Zist Chem Co., Tehran, Iran. 2.5.5. Intestinal Total Viable Bacteria (TVB) and Lactic Acid Bacteria (LAB) Counting. Subsequent to the sampling, the intestinal samples were promptly homogenized in a sterile porcelain mortar. After the preparation of the homogenate, a variety of dilutions spanning the range of $10^{-1}-10^{-7}$ were prepared by utilizing 0.9% physiological saline. A volume of 0.1 mL was extracted from the desired dilutions under sterile conditions and transferred to a plate containing nutrient agar and De Man–Rogosa–Sharpe media for the purpose of counting TVB and LAB, respectively. After 72 hr of incubation at room temperature under aerobic conditions, the colony-forming units (CFU) were counted [25].

2.6. Fish Transportation and Post-Transportation Sampling. Upon completion of the pretransportation sampling, the fish were subjected to 6-hr transportation in 60-L plastic bags. Each bag consisted of 4–5 L of water and 30 fish, which were placed to resemble a fish stocking density of 100 g/L. The bags were then filled with pure oxygen (two folds of water volume), tight sealed, and transported in a van for 6 hr. At the destination, three fish per bag were netted, anesthetized with eugenol (50 μ L/L), and euthanized by a sharp blow on the head. Subsequently, the weights of the fish were recorded, their abdominal cavities were opened, and a portion of the liver was dissected. The samples were washed with distilled water and frozen in liquid nitrogen for antioxidant parameters assays. The sampling and antioxidant analysis were carried out similar to that of before fish transportation (see above). In order to record their mortality, the remaining fish were bulk-weighed and returned to their respective tanks.

2.7. Statistical Analysis. The normal distribution of the data was determined using the Shapiro–Wilk test. The analysis of growth performance, immunological parameters, intestinal bacteria, and survivals was conducted through one-way analysis of variance (ANOVA) and Duncan tests. The analysis of hepatic antioxidant parameters was conducted through a two-way repeated-measure ANOVA and the Duncan test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22 with a significance level of 0.05.

3. Results

The 60-day rearing did not result in any mortality. Dietary phytol had a significant impact on fish growth performance and feed efficiency. As depicted in Figure 1(a)-1(e), the highest final weight and feed intake were observed in the PH-75 treatment. Also, these parameters in the PH-75 and PH-150 treatments were significantly higher than those of the CTL and PH-300 treatments. The feed intake in PH-300 treatment was significantly lower than that of CTL. The weight gain, feed efficiency, and SGR of PH-75 treatment exhibited significant elevations.

The plasma lysozyme activity significantly increased in the PH-75 treatment compared to the CTL treatment (Figure 2(a)). The P-75 and PH-150 had significantly higher plasma ACH50 activities than that of the CTL treatment



FIGURE 1: (a–e) Growth performance of Nile tilapia (mean \pm standard error) fed diets containing 0, 75, 150, and 300 mg/kg dietary phytol for 60 days. Different letters above the bars show significant differences among the treatments (n = 4; Duncan).

(Figure 2(b)). The plasma ALP activities significantly increased in the PH-75 and PH-150 treatments compared to the CTL treatment (Figure 2(c)). Phytol treatment induced no significant changes in the blood lymphocyte count (Figure 2(d)), but all phytol-treated fish had significantly higher blood neutrophil count, compared to the CTL (Figure 2(e)). In PH-300 treatment, the blood counts of WBC, monocyte, and eosinophil were significantly higher than the other treatments (Figures 2(d), 2(g), and, 2(h)).

There were no significant effects of dietary phytol on the skin mucus lysozyme, peroxidase, and ALP activities (Figure 3(a)-3(c)).

The intestinal TVB (Figure 4(a)) and LAB (Figure 4(b)) counts significantly differed among the treatments. TVB significantly decreased in PH-75 and PH-150, compared to the CTL and PH-300 treatments, while the intestinal LAB count significantly increased in the PH-75 treatment, compared to the CTL.

There was no significant difference in the fish survival among the treatments, before transportation (Figure 5(a)).

The fish survival after transportation was significantly affected by dietary phytol levels, as the highest survival was related to PH-75 and PH-150 treatments, which were significantly higher than the other treatments (Figure 5(b)).

Dietary phytol and transportation had interaction effects on the hepatic GPx (Figure 6(a)) and GR (Figure 6(b)) activities. Before transportation, all phytol-treated fish had significantly higher hepatic GPx activities, compared to the CTL, and the highest activity was related to the PH-300. Transportation led to significant elevations in the hepatic GPx activity in CTL, PH-75, and PH-150 treatments, but a decrease in PH-300 treatment. Prior to the transportation, PH-300 exhibited significantly higher hepatic GR activities in comparison to the other treatments. In CTL, PH-75, and PH-150 treatments, the hepatic GPx activity was significantly elevated, but not in the PH-300 treatment.

Dietary phytol and transportation had significant effects on the hepatic GSH (Figure 6(c)) and TBARS (Figure 6(d)) levels. The concentrations of hepatic GSH significantly increased in the PH-75 and PH-150 treatments, with the



FIGURE 2: (a–h) Plasma lysozyme, ACH50, and ALP activities and blood WBC counts of Nile tilapia (mean \pm standard error) fed diets containing 0, 75, 150, and 300 mg/kg dietary phytol for 60 days. Different letters above the bars show significant differences among the treatments (n = 4; Duncan).



FIGURE 3: (a–c) Innate immune parameters of skin mucus of Nile tilapia (mean \pm standard error) fed diets containing 0, 75, 150, and 300 mg/kg dietary phytol for 60 days. Different letters above the bars show significant differences among the treatments (n = 4; Duncan).



FIGURE 4: (a, b) Intestinal TVB and LAB of Nile tilapia (mean \pm standard error) fed diets containing 0, 75, 150, and 300 mg/kg dietary phytol for 60 days. Different letters above the bars show significant differences among the treatments (n = 4; Duncan).

highest concentration in the PH-75 treatment. Furthermore, these two treatments exhibited similar levels of hepatic TBARS, which were significantly lower than the levels observed in the other treatments. The highest hepatic TBARS level was observed in PH-300 treatment.

4. Discussion

The results indicate that dietary phytol exhibits growthpromoting properties in Nile tilapia, in contrast to those observed in common carp-fed diets containing 125–500 mg/kg phytol [16]. Hence, it appears that Nile tilapia necessitates lower concentrations of dietary phytol for growth promotion, in comparison to common carp. There are no other studies addressing this topic in fish, but dietary supplementation with aquatic plants, which are abundant in phytol, improved fish growth performance. For example, dietary fortification with common duckweed, *Lemna minor* meal [26], *Sargassum oligocystum* hot-water extract [27], and *Entromorpha intestinalis* extract [28] significantly improved the growth performance of rohu, *Labeo rohita*, Iridescent shark, *Pangasius hypophthalmus*, and electric yellow cichlid, *Labidochromis caeruleus*, respectively.

In defending the body against infections, the humoral immune system, carrying WBC and antimicrobial agents, plays critical roles. The effects of diet on fish humoral immune parameters are widely acknowledged, and certain feed additives, such as phytochemicals, have the potential to enhance these parameters [29]. Lysozyme is a natural bactericidal molecule that has the ability to directly kill Grampositive bacteria by membrane attack and indirectly kill Gram-negative bacteria by stimulating the complement system



FIGURE 5: (a, b) Survival of Nile tilapia (mean \pm standard error) fed diets containing 0, 75, 150, and 300 mg/kg dietary phytol for 60 days, before and after 6 hr transportation. Different letters above the bars show significant differences among the treatments (n = 4; Duncan).



FIGURE 6: (a–d) Hepatic antioxidant parameters of Nile tilapia (mean \pm standard error) fed diets containing 0 (CTL), 75 (PH-75), 150 (PH-150), and 300 (PH-300) mg/kg dietary phytol for 60 days, before and after 6 hr transportation (white bars: before transportation; black bars: after transportation). Different letters above the bars show significant differences among the treatments (n = 4; Duncan).

and phagocytes [30]. Furthermore, the complement proteins have diverse functions in the fish innate and adaptive immune defenses, including opsonization, cell lysis, B cell activation, and inflammation [31]. Lysozyme and complement activities exhibit remarkable elevations during fish infection to eradicate pathogens; however, certain bacteria have the ability to deactivate them [32–35]. Hence, the enhancement of plasma lysozyme and complement activities by dietary phytol may prove advantageous in safeguarding the fish against subsequent infections, as evidenced by previous studies on other feed additives [36, 37]. Similar to the present findings, common carp-fed 125–500 mg/kg dietary phytol exhibited elevations in lysozyme and complement activities [16].

Alkaline phosphatase is an essential enzyme found in lysosomes, serves as an antibacterial agent, and provides protection for fish during wound healing, parasitic infection, and stressful situations. It has an anti-inflammatory role during pathogen invasion as it dephosphrilates inflammation-triggering moieties like bacterial lipopolysaccharides [38]. In fish, neutrophils possess ALP in their lysosomes [39]. Furthermore, studies have demonstrated that dietary feed additives have the potential to enhance circulating ALP activity, subsequently leading to a higher degree of disease resistance [40–42]. Therefore, it is possible that plasma ALP may have originated from neutrophils, at least partially, which would explain the present findings.

Lymphocytes are involved in antigen production and adaptive immune responses, which are typically late but lasting responses. On the contrary, granulocytes are principally involved in the initial phase of immune defense against pathogens. Neutrophils play a significant role in the defense against fish diseases, and their proper functioning is imperative for ensuring the health of fish. They are responsible for recognizing and engulfing invading pathogens, releasing lysozyme and reactive oxygen species to kill them, and recruiting other immune cells to the site of infection [43]. Monocytes play a significant role in the immune system of fish by phagocytosing pathogens, producing inflammatory cytokines, and activating other immune cells [44]. Eosinophils are rare in fish blood, but they are involved in inflammatory responses [45]. The present results suggest that dietary phytol at 75 and 150 mg/kg may improve disease resistance of Nile tilapia, considering the higher blood neutrophil counts. The present results are in agreement with those found in other fish after treatments with different herbal additives such as Hyssop, Hyssopus officinalis, extract [46] and neem, Azadirachta indica, leaf meal [47]. On the other hand, the increase in the blood monocyte and enosinophil in PH-300 treatment can be signs of inflammation, which is supported by the highest levels of oxidative stress, mortality, and impaired antioxidant responses before/after transportation.

Skin mucosal lysozyme acts as a surface bactericidal agent [48], whereas skin mucosal peroxidase plays a role in the formation of a potent bactericidal and cytotoxic agent known as peroxidase-H₂O₂-halide complex [18]. ALP activity in the skin mucus can detoxify proinflammatory compounds generated by microbes [49]. The maintenance of strong skin mucosal immunity is imperative for the prevention of diseases in aquaculture, as water serves as the primary conduit for

pathogen transmission. A robust mucosal defense system has the ability to effectively prevent the infiltration of pathogens into the fish body [50]. The present findings indicate that phytol is incapable of inducing skin mucosal immunity, despite activating systemic immunity. However, a bath pathogenic challenge could be better reflected if dietary phytol participates in disease prevention through the stimulation of surface immunity.

The microbes present in the intestine of fish provide a multitude of advantages, including aiding in digestion by metabolizing intricate carbohydrates and proteins and facilitating their absorption [51]. They also play a crucial role in the immune system of fish by producing antimicrobial substances that safeguard the fish from harmful pathogens [52]. Overall, fish gut microbes are crucial for maintaining fish health and well-being, making them a necessary aspect of aquaculture management. The composition of the microbial population in the fish gut is influenced by diet, and phytochemicals and NICs have the potential to influence this structure, as they possess antimicrobial properties [53]. Phytol exhibits bactericidal properties against a diverse range of harmful bacteria, including Bacillus thermoamylovorans [54], Pseudomonas aeruginosa [55, 56], Escherichia coli [57], and Clostridium sporogenes, Sarcina lutea, Enterococcus faecalis [58]. Phytol has the ability to induce oxidative stress, DNA damage, and membrane damage in bacterial cells [55, 59], which explains the mechanism behind its antibacterial effect. A study has demonstrated that phytol inhibits quorum sensing and biofilm formation in Vibrio campbellii, an important aquaculture pathogen, both in vitro and in vivo. Additionally, the tomato clownfish, Amphiprion frenatus, demonstrated a higher survival rate after an experimental infection by the bacterium, attributed to lower pathogen localization [60]. In the present study, phytol exhibited a decrease in TVB levels in the intestine of tilapia, thereby demonstrating its antibacterial properties. As evidenced by this study, a reduction in TVB may provide additional resources and space for minor intestinal genera, such as LAB, to facilitate their dominance. Previous studies on this species have demonstrated that an increase in intestinal LAB has been associated with enhanced growth and health of the fish [61–63]. Therefore, it is plausible that phytol may have contributed to the intestinal and systemic health of Nile tilapia in the present study. There is currently no comparable study available for comparison, however, the utilization of dietary herbal additives such as thymol [64], common sage, Salvia officinalis [65], and Gracilaria gracilis extract [66], has had a significant impact on the diversity and structure of intestinal bacterial populations in fish.

Glutathione-related antioxidant enzymes, along with direct radical-scavenging capacity of GSH, safeguard cells against the harmful effects of oxidative stress caused by excessive reactive oxygen species [67, 68]. These antioxidants are often elevated after stressful events; stress triggers an increase in cell respiration and the production of superoxide anions, which require detoxification by superoxide dismutase [69]. The resulting hydrogen peroxide undergoes detoxification by GPx by utilizing GSH as a cofactor, resulting in GSH oxidation [67]. Subsequently, GR transforms the

oxidized GSH into its reduced form [70]. During transportation, fish may encounter various stressors, including elevated oxygen levels, fluctuations in water quality, and accumulation of water ammonia [71]; all of which can result in an increase in the production of reactive oxygen species. If the antioxidant defense system of the fish is overwhelmed, oxidative damage may occur, resulting in cellular and tissue damage, compromised immune function, and elevated susceptibility to disease [72, 73]. The present findings indicate that the mortality of the fish exhibited a pattern akin to that of lipid peroxidation, indicating that oxidative stress may be a potential cause of mortality. Phytol has been demonstrated to possess antioxidant properties [74]; a study on common carp has shown that dietary phytol can induce hepatic antioxidant enzymes and mitigate ammonia-induced lipid peroxidation [16]. It has been demonstrated that the incorporation of antioxidant phytochemicals, such as turmeric [10], or other antioxidants, such as ascorbic acid [75] and glycine [76], into the diet of fish can enhance antioxidant capacity and mitigate posttransportation oxidative stress. The antioxidant responses before and after transportation indicate that 75 and 150 mg/kg phytol can support the antioxidant system and suppress lipid peroxidation. Although dietary 300 mg/kg phytol was harmful to Nile tilapia, it suppressed the GPx and GR activities, thereby increasing lipid peroxidation.

5. Conclusion

Phytol can be a suitable feed additive for Nile tilapia, which increases growth performance, humoral immune parameters, and intestinal beneficial bacteria. Furthermore, it acts as an antioxidant agent, reducing post-transportation oxidative stress and mortality. As per the present findings, it has been determined that a dosage of 75 mg/kg phytol is suitable for Nile tilapia, whereas a dosage of 300 mg/kg should be avoided as it may result in an increase in lipid peroxidation.

Data Availability

Data are available upon a reasonable request from the corresponding author.

Ethical Approval

This study was conducted following a protocol approved by the Committee of Ethics of the Faculty of Sciences of the University of Tehran (357; 8 November 2022).

Disclosure

This study was published as a preprint version at Research Square (available at https://www.researchsquare.com/article/ rs-3327098/v1).

Conflicts of Interest

The authors have no relevant financial or nonfinancial interests to disclose.

Authors' Contributions

Saman Ahani contributed to writing–draft, methodology, analysis, and data curation. Sara Ahani contributed to writing–draft, analysis, and data curation. Morteza Yousefi contributed to conceptualization, analysis, visualization, writing–draft, and writing–editing. Ali Taheri Mirghaed contributed to conceptualization and supervision. Afaf N. Abdel Rahman contributed to conceptualization and writing–draft.

References

- A.-F. M. El-Sayed and K. Fitzsimmons, "From Africa to the world—the journey of Nile tilapia," *Reviews in Aquaculture*, vol. 15, no. S1, pp. 6–21, 2023.
- [2] H. M. R. Abdel-Latif, M. A. O. Dawood, S. Menanteau-Ledouble, and M. El-Matbouli, "The nature and consequences of co-infections in tilapia: a review," *Journal of Fish Diseases*, vol. 43, no. 6, pp. 651–664, 2020.
- [3] P. H. Serrano, Responsible Use of Antibiotics in Aquaculture, Vol. 469, Food & Agriculture Organization, Roma, Italy, 2005.
- [4] T. Defoirdt, P. Sorgeloos, and P. Bossier, "Alternatives to antibiotics for the control of bacterial disease in aquaculture," *Current Opinion in Microbiology*, vol. 14, no. 3, pp. 251–258, 2011.
- [5] N. N. Gabriel, "Review on the progress in the role of herbal extracts in tilapia culture," *Cogent Food & Agriculture*, vol. 5, no. 1, Article ID 1619651, 2019.
- [6] P. Elumalai, A. Kurian, S. Lakshmi, C. Faggio, M. A. Esteban, and E. Ringø, "Herbal immunomodulators in aquaculture," *Reviews in Fisheries Science & Aquaculture*, vol. 29, no. 1, pp. 33–57, 2021.
- [7] Á. Hernández-Contreras and M. D. Hernández, "Chapter 14 application of aromatic plants and their extracts in aquaculture," in *Feed Additives*, P. Florou-Paneri, E. Christaki, and I. Giannenas, Eds., pp. 239–259, Academic Press, 2020.
- [8] A. Ciji and M. S. Akhtar, "Stress management in aquaculture: a review of dietary interventions," *Reviews in Aquaculture*, vol. 13, pp. 2190–2247, 2021.
- [9] T. P. Boaventura, C. F. Souza, A. L. Ferreira et al., "The use of Ocimum gratissimum L. essential oil during the transport of Lophiosilurus alexandri: water quality, hematology, blood biochemistry and oxidative stress," Aquaculture, vol. 531, Article ID 735964, 2021.
- [10] S. M. Hoseini, S. K. Gupta, M. Yousefi et al., "Mitigation of transportation stress in common carp, *Cyprinus carpio*, by dietary administration of turmeric," *Aquaculture*, vol. 546, Article ID 737380, 2022.
- [11] M. Yousefi, S. M. Hoseini, R. A. Weber et al., "Alleviation of transportation-induced stress in Nile tilapia, *Oreochromis niloticus*, using brackish water," *Aquaculture Reports*, vol. 27, Article ID 101378, 2022.
- [12] F. S. Zanuzzo, R. E. Sabioni, L. N. F. Montoya, G. Favero, and E. C. Urbinati, "Aloe vera enhances the innate immune response of pacu (*Piaractus mesopotamicus*) after transport stress and combined heat killed *Aeromonas hydrophila* infection," *Fish & Shellfish Immunology*, vol. 65, pp. 198–205, 2017.
- [13] J. M. G. Beltrán and M. Á. Esteban, "Nature-identical compounds as feed additives in aquaculture," *Fish & Shellfish Immunology*, vol. 123, pp. 409–416, 2022.
- [14] J. de Moraes, R. N. de Oliveira, J. P. Costa et al., "Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease *Schistosomiasis mansoni*," *PLoS Neglected Tropical Diseases*, vol. 8, no. 1, Article ID e2617, 2014.

- [15] M. Saha and P. K. Bandyopadhyay, "In vivo and in vitro antimicrobial activity of phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* nees. (Acanthaceae), to control severe bacterial disease of ornamental fish, *Carassius auratus*, caused by *Bacillus licheniformis* PKBMS₁₆," *Microbial Pathogenesis*, vol. 141, Article ID 103977, 2020.
- [16] S. M. Hoseini, B. Gharavi, A. T. Mirghaed, S. H. Hoseinifar, and H. Van Doan, "Effects of dietary phytol supplementation on growth performance, immunological parameters, antioxidant and stress responses to ammonia exposure in common carp, *Cyprinus carpio* (Linnaeus, 1758)," *Aquaculture*, vol. 545, Article ID 737151, 2021.
- [17] AOAC, Official Methods of Analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists, Washington, DC, USA, 2005.
- [18] F. A. Guardiola, A. Cuesta, M. Arizcun, J. Meseguer, and M. A. Esteban, "Comparative skin mucus and serum humoral defence mechanisms in the teleost gilthead seabream (*Sparus aurata*)," *Fish & Shellfish Immunology*, vol. 36, no. 2, pp. 545– 551, 2014.
- [19] J. Dacie and S. Lewis, *Practical Hematology*, Charchill and Livingston, London, 1996.
- [20] P. C. Blaxhall, "The haematological assessment of the health of freshwater fish," *Journal of Fish Biology*, vol. 4, pp. 593–604, 1972.
- [21] A. E. Ellis, "Lysozyme assays," in *Techniques in Fish Immunology*, J. S. Stolen, Ed., pp. 101–103, SOS Publication, Fair Haven, 1990.
- [22] M. Esmaeili, A. A. Kenari, and A. N. Rombenso, "Effects of fish meal replacement with meat and bone meal using garlic (*Allium sativum*) powder on growth, feeding, digestive enzymes and apparent digestibility of nutrients and fatty acids in juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)," *Aquaculture Nutrition*, vol. 23, pp. 1225–1234, 2017.
- [23] T. Yano, "Assays of hemolytic complement activity," in *Techniques in Fish Immunology*, J. S. Stolen, Ed., pp. 131–141, SOS publication, Fair Haven, 1992.
- [24] M. J. Quade and J. A. Roth, "A rapid, direct assay to measure degranulation of bovine neutrophil primary granules," *Veterinary Immunology and Immunopathology*, vol. 58, no. 3-4, pp. 239–248, 1997.
- [25] S. Alonso, M. Carmen Castro, M. Berdasco, I. G. de la Banda, X. Moreno-Ventas, and A. H. de Rojas, "Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture," *Probiotics and Antimicrobial Proteins*, vol. 11, pp. 569–579, 2019.
- [26] R. K. Goswami, A. K. Shrivastav, J. G. Sharma, D. R. Tocher, and R. Chakrabarti, "Growth and digestive enzyme activities of rohu labeo rohita fed diets containing macrophytes and almond oil-cake," *Animal Feed Science and Technology*, vol. 263, Article ID 114456, 2020.
- [27] F. N. Baleta and J. M. Bolaños, "Growth and immune response of *Pangasius hypophthalmus* fed diets containing seaweed extracts as immunostimulant," *Brazilian Archives of Biology* and *Technology*, vol. 62, Article ID e19180083, 2019.
- [28] F. Pezeshk, S. Babaei, A. A. Kenari, M. Hedayati, and M. Naseri, "The effect of supplementing diets with extracts derived from three different species of macroalgae on growth, thermal stress resistance, antioxidant enzyme activities and skin colour of electric yellow cichlid (*Labidochromis caeruleus*)," *Aquaculture Nutrition*, vol. 25, pp. 436–443, 2019.

- [29] M. A. Dawood, S. Koshio, and M. Á. Esteban, "Beneficial roles of feed additives as immunostimulants in aquaculture: a review," *Reviews in Aquaculture*, vol. 10, pp. 950–974, 2018.
- [30] Q. Song, Y. Xiao, Z. Xiao et al., "Lysozymes in fish," Journal of Agricultural and Food Chemistry, vol. 69, no. 50, pp. 15039– 15051, 2021.
- [31] L. Bavia, L. E. Santiesteban-Lores, M. C. Carneiro, and M. M. Prodocimo, "Advances in the complement system of a teleost fish, *Oreochromis niloticus*," *Fish & Shellfish Immunology*, vol. 123, pp. 61–74, 2022.
- [32] D.-D. Chen, J.-H. Li, Y.-Y. Yao, and Y.-A. Zhang, "Aeromonas hydrophila suppresses complement pathways via degradation of complement C3 in bony fish by metalloprotease," Fish & Shellfish Immunology, vol. 94, pp. 739–745, 2019.
- [33] W. El-Houseiny, M. F. Mansour, W. A. M. Mohamed et al., "Silver nanoparticles mitigate Aeromonas hydrophila-induced immune suppression, oxidative stress, and apoptotic and genotoxic effects in Oreochromis niloticus," Aquaculture, vol. 535, Article ID 736430, 2021.
- [34] A. Das, P. K. Sahoo, B. R. Mohanty, and J. K. Jena, "Pathophysiology of experimental *Aeromonas hydrophila* infection in *Puntius sarana*: early changes in blood and aspects of the innate immune-related gene expression in survivors," *Veterinary Immunology and Immunopathology*, vol. 142, no. 3-4, pp. 207–218, 2011.
- [35] B. Liu, X. Ge, J. Xie et al., "Effects of anthraquinone extract from *Rheum officinale* Bail on the physiological responses and HSP70 gene expression of *Megalobrama amblycephala* under *Aeromonas hydrophila* infection," *Fish & Shellfish Immunology*, vol. 32, no. 1, pp. 1–7, 2012.
- [36] T. Q. Nhu, B. T. Bich Hang, L. T. Bach et al., "Plant extractbased diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*)," *Fish & Shellfish Immunology*, vol. 92, pp. 913–924, 2019.
- [37] J. Zhou, X.-L. Song, J. Huang, and X.-H. Wang, "Effects of dietary supplementation of A3α-peptidoglycan on innate immune responses and defense activity of Japanese flounder (*Paralichthys olivaceus*)," Aquaculture, vol. 251, no. 2–4, pp. 172–181, 2006.
- [38] A. Presbitero, E. Mancini, R. Brands, V. V. Krzhizhanovskaya, and P. M. A. Sloot, "Supplemented alkaline phosphatase supports the immune response in patients undergoing cardiac surgery: clinical and computational evidence," *Frontiers in Immunology*, vol. 9, Article ID 2342, 2018.
- [39] A. Azevedo and L. O. Lunardi, "Cytochemical characterization of eosinophilic leukocytes circulating in the blood of the turtle (*Chrysemys dorbignih*)," *Acta Histochemica*, vol. 105, no. 1, pp. 99–105, 2003.
- [40] S. T. Singh, D. Kamilya, B. Kheti, B. Bordoloi, and J. Parhi, "Paraprobiotic preparation from *Bacillus amyloliquefaciens* FPTB16 modulates immune response and immune relevant gene expression in *Catla catla* (Hamilton, 1822)," *Fish & Shellfish Immunology*, vol. 66, pp. 35–42, 2017.
- [41] C.-N. Zhang, X.-F. Li, W.-N. Xu et al., "Combined effects of dietary fructooligosaccharide and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*)," *Fish & Shellfish Immunology*, vol. 35, no. 5, pp. 1380–1386, 2013.
- [42] M. Abdel-Tawwab, I. Adeshina, A. Jenyo-Oni, E. K. Ajani, and B. O. Emikpe, "Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to

dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection," *Fish & Shellfish Immunology*, vol. 78, pp. 346–354, 2018.

- [43] J. J. Havixbeck, A. M. Rieger, M. E. Wong, J. W. Hodgkinson, and D. R. Barreda, "Neutrophil contributions to the induction and regulation of the acute inflammatory response in teleost fish," *Journal of Leukocyte Biology*, vol. 99, no. 2, pp. 241–252, 2016.
- [44] X.-J. Lu and J. Chen, "Specific function and modulation of teleost monocytes/macrophages: polarization and phagocytosis," *Zoological Research*, vol. 40, no. 3, pp. 146–150, 2019.
- [45] A. J. Ainsworth, "Fish granulocytes: morphology, distribution, and function," *Annual Review Fish Diseases*, vol. 2, pp. 123– 148, 1992.
- [46] M. Yousefi, S. M. Hoseini, B. Abtahi, Y. A. Vatnikov, E. V. Kulikov, and R. N. Yurievna, "Effects of dietary methanolic extract of hyssop, *Hyssopus officinalis*, on growth performance, hepatic antioxidant, humoral and intestinal immunity, and intestinal bacteria of rainbow trout, *Oncorhynchus mykiss*," *Frontiers in Marine Science*, vol. 9, Article ID 1026651, 2022.
- [47] A. D. Talpur and M. Ikhwanuddin, "Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with Vibrio harveyi," Fish & Shellfish Immunology, vol. 34, no. 1, pp. 254–264, 2013.
- [48] L. Li, J. C. R. Cardoso, R. C. Félix, A. P. Mateus, A. V. M. Canário, and D. M. Power, "Fish lysozyme gene family evolution and divergent function in early development," *Developmental and Comparative Immunology*, vol. 114, Article ID 103772, 2021.
- [49] J.-P. Lallès, "Biology, environmental and nutritional modulation of skin mucus alkaline phosphatase in fish: a review," *Fish & Shellfish Immunology*, vol. 89, pp. 179–186, 2019.
- [50] E. J. Noga, Fish Disease: Diagnosis and Treatment, John Wiley & Sons, Amnes, Iowa, 2011.
- [51] S. M. Hoseini, M. Yousefi, A. Afzali-Kordmahalleh, E. Pagheh, and A. Taheri Mirghaed, "Effects of dietary lactic acid supplementation on the activity of digestive and antioxidant enzymes, gene expressions, and bacterial communities in the intestine of common carp, *Cyprinus carpio*," *Animals*, vol. 13, no. 12, Article ID 1934, 2023.
- [52] G. D. Gómez and J. L. Balcázar, "A review on the interactions between gut microbiota and innate immunity of fish," *FEMS Immunology and Medical Microbiology*, vol. 52, no. 2, pp. 145–154, 2007.
- [53] H. M. Abdel-Latif, S. Yilmaz, and D. Kucharczyk, "Functionality and applications of phytochemicals in aquaculture nutrition," *Front in Veterinary Science*, vol. 10, Article ID 1218542, 2023.
- [54] B. Petpheng, B. Mudtaleb, W. Piboon, N. Paichid, and K. Sangkharak, "The extraction of phytol from *Hydrilla verticillata* using ultrasonic-assisted extraction (UAE), the analysis of antibacterial activity, and the utilization of residual extract for polyhydroxyalkanoate (PHA) production," *Biomass Conversion and Biorefinery*, 2023.
- [55] W. Lee, E.-R. Woo, and D. G. Lee, "Phytol has antibacterial property by inducing oxidative stress response in *Pseudomonas aeruginosa*," *Free Radical Research*, vol. 50, pp. 1309– 1318, 2016.
- [56] B. Pejin, A. Ciric, J. Glamoclija, M. Nikolic, and M. Sokovic, "In vitro anti-quorum sensing activity of phytol," *Natural Product Research*, vol. 29, no. 4, pp. 374–377, 2015.

- [57] M. T. Ghaneian, M. H. Ehrampoush, A. Jebali, S. Hekmatimoghaddam, and M. Mahmoudi, "Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant," *Environmental Health Engineering Management Journal*, vol. 2, pp. 13–16, 2015.
- [58] B. Pejin, T. Kartali, B. Stanimirović, and M. Karaman, "Phytol may inspire new medicinal foods for the treatment of heart disease," *Asian Journal of Chemistry*, vol. 26, pp. 8261-8262, 2014.
- [59] F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar, "Biological effects of essential oils—a review," *Food and Chemical Toxicology*, vol. 46, no. 2, pp. 446–475, 2008.
- [60] R. Alexpandi, G. Abirami, L. Satish et al., "Tocopherol and phytol possess anti-quorum sensing mediated anti-infective behavior against *Vibrio campbellii* in aquaculture: an *in vitro* and *in vivo* study," *Microbial Pathogenesis*, vol. 161, no. Pt A, Article ID 105221, 2021.
- [61] G. F. Alves Jesus, M. S. Owatari, S. A. Pereira et al., "Effects of sodium butyrate and *Lippia origanoides* essential oil blend on growth, intestinal microbiota, histology, and haematoimmunological response of Nile tilapia," *Fish & Shellfish Immunology*, vol. 117, pp. 62–69, 2021.
- [62] N. Pirarat, T. Kobayashi, T. Katagiri, M. Maita, and M. Endo, "Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*)," *Veterinary Immunology and Immunopathology*, vol. 113, no. 3-4, pp. 339–347, 2006.
- [63] S. Boonanuntanasarn, N. Tiengtam, T. Pitaksong, P. Piromyou, and N. Teaumroong, "Effects of dietary inulin and Jerusalem artichoke (*Helianthus tuberosus*) on intestinal microbiota community and morphology of Nile tilapia (*Oreochromis niloticus*) fingerlings," *Aquaculture Nutrition*, vol. 24, pp. 712–722, 2018.
- [64] A. S. Abd El-Naby, A. A. Al-Sagheer, S. S. Negm, and M. A. E. Naiel, "Dietary combination of chitosan nanoparticle and thymol affects feed utilization, digestive enzymes, antioxidant status, and intestinal morphology of *Oreochromis niloticus*," *Aquaculture*, vol. 515, Article ID 734577, 2020.
- [65] E. E. Hussein, M. F. El Basuini, A. M. Ashry et al., "Effect of dietary sage (*Salvia officinalis* L.) on the growth performance, feed efficacy, blood indices, non-specific immunity, and intestinal microbiota of European sea bass (*Dicentrarchus labrax*)," *Aquaculture Reports*, vol. 28, Article ID 101460, 2023.
- [66] A. T. Gonçalves, M. Simões, C. Costa, R. Passos, and T. Baptista, "Modulatory effect of *Gracilaria gracilis* on European seabass gut microbiota community and its functionality," *Scientific Reports*, vol. 12, no. 1, Article ID 14836, 2022.
- [67] R. F. Burk and K. E. Hill, "4.13—glutathione peroxidases," in *Comprehensive Toxicology*, C. A. McQueen, Ed., pp. 229–242, Elsevier, Oxford, (Second) edition, 2010.
- [68] J.-D. Liu, C. Chi, X.-C. Zheng et al., "Effect of dietary glutathione supplementation on the immune responses and the fatty acid and amino acid composition in Chinese mitten crab, *Eriocheir sinensis*," *Aquaculture Reports*, vol. 15, Article ID 100217, 2019.
- [69] M. N. Islam, A. Rauf, F. I. Fahad et al., "Superoxide dismutase: an updated review on its health benefits and industrial applications," *Critical Reviews in Food Science and Nutrition*, vol. 62, no. 26, pp. 7282–7300, 2022.
- [70] N. Couto, J. Wood, and J. Barber, "The role of glutathione reductase and related enzymes on cellular redox homoeostasis

network," *Free Radical Biology & Medicine*, vol. 95, pp. 27–42, 2016.

- [71] T. S. Harmon, "Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics," *Reviews in Aquaculture*, vol. 1, pp. 58–66, 2009.
- [72] J. D. Biller and L. S. Takahashi, Oxidative Stress and Fish Immune System: Phagocytosis and leukocyte Respiratory Burst Activity, vol. 90, pp. 3403–3414, Anais da Academia Brasileira de Ciencias, 2018.
- [73] K. V. Brix, G. De Boeck, S. Baken, and D. J. Fort, "Adverse outcome pathways for chronic copper toxicity to fish and amphibians," *Environmental Toxicology and Chemistry*, vol. 41, no. 12, pp. 2911–2927, 2022.
- [74] M. T. Islam, E. S. Ali, S. J. Uddin et al., "Phytol: a review of biomedical activities," *Food and Chemical Toxicology*, vol. 121, pp. 82–94, 2018.
- [75] S. Peng, Z. Shi, Y. Fei, Q. Gao, P. Sun, and J. Wang, "Effect of high-dose vitamin C supplementation on growth, tissue ascorbic acid concentrations and physiological response to transportation stress in juvenile silver pomfret, *Pampus* argenteus," Journal of Applied Ichthiology, vol. 29, pp. 1337– 1341, 2013.
- [76] S. M. Hoseini, N. Majidiyan, A. T. Mirghaed, S. H. Hoseinifar, and H. Van Doan, "Dietary glycine supplementation alleviates transportation-induced stress in common carp, *Cyprinus carpio*," *Aquaculture*, vol. 551, Article ID 737959, 2022.