Aquaculture Reports 36 (2024) 102154

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

A commercial seaweed extract increases growth performance, immune responses, and related gene expressions in whiteleg shrimp (*Litopenaeus vannamei*)

Mohamed Ashour [a,1,*], Ahmed Said Al-Souti [b,2,*], Mohamed M. Mabrouk [c], Mohammed A.E. Naiel [d], Elsayed M. Younis [e], Abdelwahab A. Abdelwarith [e], Marwa F. AbdEl-Kader [f], Mohammad Shaaban Abu Husein [g], Abeer El saharty [a], Mohamed A. Elokaby [a], Ahmed I.A. Mansour [a], Ahmed A.A. El-Bahlol [c], Einar Ringø [h], Ehab El-Haroun [i], Ahmed F. Abdelhamid [c]

[a] *National Institute of Oceanography and Fisheries (NIOF), Cairo 11516, Egypt*
[b] *AL Hail Aquaculture Unit, Department of Marine Science, Fisheries College of Agriculture and Marine Science, Sultan Qaboos University, Muscat 123, Oman*
[c] *Department of Fish Production, Faculty of Agriculture in Cairo, Al-Azhar University, Cairo 11884, Egypt*
[d] *Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt*
[e] *Department of Zoology, College of Science, King Saudi University, PO Box 2455, Riyadh 11451, Saudi Arabia*
[f] *Department of Fish Health and Management, Sakha Aquaculture Research Unit, Central Laboratory for Aquaculture Research, A.R.C, Kafrelsheikh, Egypt*
[g] *Zoology Department, Marine Biology Branch, Faculty of Science, Al-Azhar University, Cairo 11884, Egypt*
[h] *Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries, and Economics, UiT The Arctic University of Norway, Tromsø N-9037, Norway*
[i] *Fish Nutrition Research Laboratory, Animal Production Department, Faculty of Agriculture, Cairo University, Cairo 12613, Egypt*

ARTICLE INFO

Keywords:
TAM
Seaweed phytochemicals
L. vannamei
Growth Performance
Feed Utilisation
Antioxidant capacity
Immune and gene expression

ABSTRACT

Seaweed liquid extracts are an attractive source of phytochemicals with high potential applicability in the aquafeed-additive industry. A commercial seaweed liquid extract (True Algae Max, TAM®), which has a marine seaweed odor, also dislplays significant levels of polysaccharides, phytochemicals, phenolic, and flavonoid compounds showing antioxidant activities and DPPH inhibition. This study investigates the impact of diets supplemented with TAM® as a functional additive on the growth, nutrient utilization, immune responses, and immune-related gene expressions of whiteleg shrimp (*Litopenaeus vannamei*). A total of 750 postlarvae (PL, with an average initial weight of 0.053±0.001 g) were divided into five experimental groups, comprising three replicates per dietary treatment. For an eight-week experimental period, all groups were fed identical diets except for the variation in TAM® inclusion levels. The basal diet (control diet) had no inclusion level of TAM® ($TAM_{0\%}$). Groups 2–5 each contain TAM® inclusion at levels of 1% (10 mL gk^{-1} diet $TAM_{1\%}$), 2% (20 mL gk^{-1} diet $TAM_{2\%}$), 3% (30 mL gk^{-1} diet $TAM_{3\%}$), and 4% (40 mL gk^{-1} diet $TAM_{4\%}$), respectively. The results concluded that TAM® has great potential as a feed additive for whiteleg shrimp, compared to the control group. The group $TAM_{2\%}$ significantly achieved final weight (4.337 g), weight gain (4.287 g) specific growth rate (3.423% / day), feed conversion ratio (1.970), feed efficiency ratio (0.507), and protein efficiency ratio (1.407), compared to the control group (3.900 g, 3.850 g, 3.347%/day, 2.183, 0.458, and 1.270, respectively). Whole-body composition of protein and lipid contents were significantly improved by all TAM group including $TAM_{2\%}$ (51.18% and 4.49%, respectively), compared to the control group (49.38% and 4.15%, respectively). As well as, shrimp in group $TAM_{2\%}$ achieved the highest values of lysozyme (3.92 μg mL^{-1}), superoxide dismutase (SOD, 11.92 IU mL^{-1}), catalase (CAT, 13.04 IU g^{-1}), lipase (31.24 IU L^{-1}), and amylase (31.24 IU L^{-1}), compared to the control group (3.19 μg mL^{-1}, 8.80 IU mL^{-1}, 10.84 IU g^{-1}, 17.35 IU L^{-1}, 21.65 IU L^{-1}, respectively). For gene expressions experiment, four immune-related were performed in this study; Peroxiredoxin (*Prx*), Prophenoloxidase (*PPO1*), P53-like protein isoform delta (*p53*), and Hemocyanin subunit L5 (*L5H*). The results showed that shrimp in group

* Corresponding authors.
E-mail addresses: microalgae_egypt@yahoo.com (M. Ashour), souti@squ.edu.om (A.S. Al-Souti).
[1] https://www.scopus.com/authid/detail.uri?authorId=57201774072
[2] https://www.scopus.com/authid/detail.uri?authorId=54987363800

https://doi.org/10.1016/j.aqrep.2024.102154
Received 5 November 2023; Received in revised form 6 May 2024; Accepted 15 May 2024
Available online 22 May 2024

$TAM_{2\%}$ achieved the highest significant *P53* gene transcription compared to the control group or the other TAM groups. Compared to other groups, the $TAM_{1\%}$ group considerably increased *PPO1* gene transcription, while $TAM_{3\%}$ displayed the greatest *Prx* gene transcription. On the other hand, compared to control group, all TAM groups significantly improved *L5H* gene transcription. In conclusion, the current study revealed that TAM® diet supplementation (20 mL of TAM® per kg diet) represents a promising, eco-friendly, and sustainable feed additive in the shrimp aquadiet industry.

1. Introduction

Recently, the shrimp aquaculture sector has experienced remarkable growth and has become one of the most critical contributers in world aquaculture production (Gillett, 2008; Ahmed et al., 2019; Abbas et al., 2020). The Pacific whiteleg shrimp (*Litopenaeus vannamei*) has emerged as the most commonly cultivated penaeid species, accounting for more than 70% of the world's shrimp aquaculture output. This species is preferred because of its fast growth rate, high reproductive capacity, and can tolerate a wide range of salinity levels (Li et al., 2018; Lukwambe et al., 2019). One of the most significant problems is the feed quality and meeting nutritional specifications, which affects the growth and overall health of the shrimp. Another challenge is low survivability rates, caused by diseases, poor water quality, and environmental stressors (Abo-Taleb et al., 2020a; Anh et al., 2021; El-Sayed, 2021; Emerenciano et al., 2022). Additionally, environmental pollution and climate change pose significant threats, impacting the sustainability of aquaculture, fisheries, aquatic habitats, and aquatic organisms (Abo-Taleb et al., 2020b; Abo-Taleb et al., 2021; Magouz et al., 2021). These issues need to be addressed through sustainable and environmentally friendly practices to ensure the long-term viability of the industry (Ahmed and Turchini, 2021; Iber and Kasan, 2021; Abisha et al., 2022; Mansour et al., 2022a).

Due to the increasing demand for shrimp products globally, the shrimp feed industry has been resolving to provide effective solutions to various challenges (El-Sayed, 2021). To address these issues, the industry has implemented selective strategies with one of the most important being feed additive supplementation. This approach involves the addition of various compounds to shrimp feeds to improve growth, boost immunity, and enhance disease resistance. These dietary additives and supplements can include vitamins, minerals, amino acids, probiotics, prebiotics, and other bioactive compounds, such as plant extracts (Sharawy et al., 2022). Shrimp rely on and innate immunity, which includes cellular and humoral responses, to protect against pathogens In addition to growth and immunity, the quality of shrimp feed additives can also be assessed through their impact on gene expression and immunological indices associated with immune mechanisms and the mitigation of oxidative stress biochemistry and physiology (Fawzy et al., 2022; Hu, 2022; Zhang et al., 2022).

Immunological indices, such as phagocytic activity and lysozyme activity, can also be used to evaluate the effectiveness of shrimp feed additives by improving immunity (Hu, 2022). The bioactive compound content and potency of feed additives is another critical factor that affects their quality. The composition of the feed additive can impact the nutrient profile of the shrimp and influence their growth and development status. Another critical consideration in our understanding of shrimp feed additives is their environmental impact. The production and use of feed additives can have significant environmental implications, including nutrient pollution, water quality degradation, and greenhouse gas emissions (Alprol et al., 2021). Seaweed is a marine aquatic plant naturally rich in several phytochemical compounds that have a plethora of industrial applications (Alam et al., 2013; Osman et al., 2013; Paradiković et al., 2019; Torres et al., 2019; Elshobary et al., 2020; Alprol et al., 2021; Ashour et al., 2021a). The shores of Egypt along the Mediterranean are home to a variety of wild seaweed genera that can be sustainably harvested throughout the year. The most common seaweed species reported along Alexandria's Mediterranean coast are *Pterocladiella capillacea, Jania rubens* (both red macroalgae), and *Ulva lactuca* (green macroalgae). These seaweeds have been studied for their biochemistry, mineral content, antioxidant activity, as well as their ability to inhibit *in vivo* microbial growth (El Nemr et al., 2012; El-Said and El-Sikaily, 2013; Khaled et al., 2014; Hassan and Shobier, 2018).

For aquaculture purposes, several studies showed that seaweed extracts are a valuable resource as they contain a range of biomolecules and nutrients, such as polysaccharides, peptides, phenolic compounds, and pigments that can improve growth, nutrient absorption, immune function, and gene expression of cultured aquatic animals (Ashour et al., 2020b; Ashour et al., 2021b; Mansour et al., 2022b). Moreover, seaweed extracts have also been revealed to have antimicrobial and antioxidant properties, help to reduce the risk of disease outbreaks, and improve the overall health of several aquatic animal species. Moreover, in Nile tilapia (*Oreochromis niloticus*) culture, a commercial seaweed extract (TAM®) has previously been reported as an attractive aquafeed additive (Ashour et al., 2020b) and water quality enhancer (Ashour et al., 2021b). The current study served to evaluate the impact of the different inclusion levels (0, 1, 2, 3, and 4%) of this selected commercial seaweed liquid extract (True Algae Max, TAM®) into shrimp diets; the major objectives being to quantify effects on growth performance, nutrient utilization, immune responses, and related-gene expressions of the whiteleg shrimp, *Litopenaeus vannamei*.

2. Materials and Methods

2.1. Seaweed

The commercial seaweed liquid extract (True Algae Max, TAM®) has been previously submitted as a patent (Ashour, 2019), and the production methodology of TAM® was earlier described by Ashour et al. (2020a). In brief, two Rhoophyceae (*Pterocladiella capillacea* and *Jania rubens*) and one Chlorophyceae species (*Ulva lactuca*) were harvested from rocky sites on the Mediterranean Coast of Alexandria, Egypt (31° 16′ 16.0″ N, 30° 10′ 28.0″ E) to be used in preparing and producing TAM®. After harvesting, the impurities were removed, then the algae were washed, air-dried, powdered, and kept in bags at room temperature, 22 °C, until the TAM® preparation and analysis. Phytochemical, biochemical, chemical, and physical analyses of crude TAM® were determined as described previously (Ashour et al., 2020b; Alprol et al., 2021). TAM® physical analyses showed that TAM®, which has a dark-brown color and a marine seaweed odor, revealed a density and pH value of 1.2 and 9–9.5, respectively. The biochemical analyses (% DM) of total polysaccharides, total organic matter, and total dissolved solids values were 15%, 8.2%, and 2.6%, respectively. Moreover, the phytochemical analyses (% DM) of DPPH inhibition (70.33%), total antioxidant capacity (54.52 mg g^{-1}), total phenolic compounds (101.67 mg g^{-1}), total flavonoid compounds (2.60 mg g^{-1}), and total ascorbic acid (1.66 mg g^{-1}) were determined according to previous studies (Ashour et al., 2020b; Ashour et al., 2023). Chemical analyses (major nutrients, minor nutrients, and heavy metals) revealed major nutrient (%) values (%) of K, P, and N were 12%, 2.4%, and 0.14%, respectively, while the minor nutrient values (ppm) (%) of Cu, Fe, Mg, Zn, and Mn values were 0.39 ppm, 16.18 ppm, 19.72 ppm, 1.19 ppm, and 3.72 ppm, respectively. According to previous studies, no amounts of heavy metals of Cd, Cr, Pb, and Ni were reported, while As was reported as traces (0.55 ppm) (Ashour et al., 2020b; Alprol et al., 2021). The phytochemical profile of TAM® was performed by GC-Mass as

described elsewhere (Ashour et al., 2020b), with the important biological activities according to the literature presented in Table 1.

2.2. Shrimp

Shrimp postlarvae (PLs) were procured from a private hatchery in Kafr El-Sheikh City, Egypt, and carefully transferred to the Fish Nutrition Laboratory, Baltim Research Station, National Institute of Oceanography and Fisheries (NIOF), Kafr El-Sheikh, Egypt. The PLs were allowed to acclimate to the experimental conditions for two weeks in concrete tanks (5 m × 5 m × 1 m), with a water temperature of 26 ± 2 °C, dissolved oxygen (DO) of 5 mg L^{-1} (by continuous aeration), and were fed a commercial basal diet of 45% protein (Table 2), four times daily, until the larvae reached full satiation. The tanks used for acclimatization received groundwater with a salinity of 40 ppt mixed with freshwater to balance the water entering the shrimp ponds, which had a salinity of 26 ± 1 ppt prior to the experimental start.

Table 1
Phytochemical profile of TAM®, with the most important biological activities according to the literature.

Compound Name	Nature	Biological impacts	Refs.
5-Silaspiro[4.4] nona-1,3,6,8-tetraene,3,8-bis (diet-hylboryl)-2,7-diethyl-1,4,6,9-tetraphenyl-	Silicon-boron compound	Fish and plant growth regulator and immunity enhancer	(Mekki, 2015; Laane, 2018; **Ashour et al., 2020**)
Nonadecane	Alkane	Fish and plant immunity enhancer; antioxidant; antimicrobial; anti-inflammatory,	(**Ibrahim et al., 2016; Abdel-Latif et al., 2018; Mohy El-Din and Mohyeldin, 2018; WM Hassan and H Shobier, 2018; Ashour et al., 2020**)
Rhodopin	Carotenoid	Fish and plant growth enhancer; antioxidant	(**El-Din and El-Ahwany, 2016; Ashour et al., 2020**)
Milbemycin-oxime	Macrocyclic lactones	Fish and plant immunity enhancer; antiparasitic; antihelmintic; insecticidal	(**Takiguchi et al., 1980; Prichard et al., 2012; Kumar et al., 2014; Ashour et al., 2020**)
Tridecanoic acid methyl ester	Fatty acid methyl esters (FAMEs)	Antioxidant; herbicidal; antimicrobial; surfactants	(**Azam et al., 2005; Agoramoorthy et al., 2007; Synowiec et al., 2017; Ashour et al., 2020**)
9,12-Octadecadienoic acid methyl ester, (*E*, *E*)-	FAMEs	Antioxidant; herbicidal; antimicrobial; surfactants	(**Azam et al., 2005; Agoramoorthy et al., 2007; Synowiec et al., 2017; Ashour et al., 2020**)
γ-Linolenic acid methyl ester	FAMEs	Antioxidant; herbicidal; antimicrobial;	(**Azam et al., 2005; Agoramoorthy et al., 2007; Synowiec et al., 2017; Ashour et al., 2020**)
Oleic Acid	Fatty acid	Fish and plant immunity enhancer; anti-inflammatory	(**Matanjun et al., 2009; Vassiliou et al., 2009; Cardoso et al., 2017; Ashour et al., 2020**)
Phytol	Diterpene alcohol	Antioxidant; plant growth enhancer	(**Santos et al., 2013; Gutbrod et al., 2019; Ashour et al., 2020**)

Table 2
The formulation and chemical composition of the basal diet (dry matter basis).

Ingredient	Experimental diet (%)
Fish meal Peru[a]	12
Fish meal Ca Mau[b]	18
Wheat gluten[c]	3
Defatted soybean meal[d]	28
Squid liver powder[e]	5
Wheat flour	25
Fish oil	3
Lecithin	1
Binder (GG)[f]	0.5
Cholesterol	0.1
Choline chloride 60%	0.6
MCP	0.3
Premix[g]	2.0
Ascorbic Acid	0.1
Gelatin	1.0
Lysine	0.1
Methionine	0.3
Total (%)	100
Chemical composition	%
Crude Protein	45.4
Crude lipid	7.82
Carbohydrate	33.98
Crude Fiber	3.63
Ash	9.17
Gross energy (MJ kg^{-1})	19.9

[a] Peruvian fishmeal, Pesquera Exalmar (CP 65%); [b] Eco-Fish Ca Mau Viet Nam (CP 60%); [c] VMC Group Vietnam; [d] Maharashtra Solvent Extraction LTD India; [e] An Giang Agriculture and Foods import-export joint stock company; [f] Binder (Guar gum) was imported from Pakistan and supplied by Hoa Chat Can Tho Comp, Vietnam; and [g] Premix was provided by DSM, Germany.

2.3. Diets

Five experimental diets (groups) were formulated to be isonitrogenous (54.4% crude protein), and isocaloric (19.9 MJ kg^{-1} gross energy, DE). All groups were fed identical diets except for the variation in seaweed liquid extract (TAM®) inclusion levels, which was prepared as previously described (Ashour et al., 2020b). The basal diet (control diet) was not supplemented with TAM® ($TAM_{0\%}$). Groups 2–5 were added TAM at levels of 1% (10 mL gk^{-1} diet $TAM_{1\%}$), 2% (20 mL gk^{-1} diet $TAM_{2\%}$), 3% (30 mL gk^{-1} diet $TAM_{3\%}$), and 4% (40 mL gk^{-1} diet $TAM_{4\%}$), respectively. The diets were performed by blended the dry ingredients were together to create a homogenous mixture. Using the Sprout-Waldron Laboratory Pellet Mill (CPM, California Pellet Mill Co., USA), pellets of 2 mm in size were produced. The temperature during the pelleting process was kept below 40 °C. To incorporate seaweed extract (TAM), the crude extract was dissolved in distilled water and sprayed onto the surface of the diet, as described by Mehrabi et al. (2012). The control diet (TAM0%) received an equal amount of distilled water without TAM. All diets were then dried at 40 °C for 48 hours, resulting in a moisture content of approximately 10%. Subsequently, a 5 mL kg−1 diet of sunflower oil was sprayed onto the basal diet to cover the aqueous extract (Zeraatpisheh et al., 2018). Finally, the diets were air dried for 4 hours and packed in cellophane bags. Prior to use, they were cooled at 4 °C. The biochemical analysis of the control diet and feed diet ingredients are shown in Table 2. Gross energy (GE) contents were calculated according to the gross caloric values by Jobling (1983).

2.4. Culture Technique and Water Quality

In this study, a total of 750 PLs were used for the five treatments. After 15 days of acclimation, 50 PLs was stocked into one net hapa (0.7 × 0.7 × 1 m) at a total of 150 PLs for each group, three replicates for each group. The hapa net was fixed in concrete ponds (4 × 2 × 1 m). During the eight weeks experiment, PLs were reared under the conditions of photoperiod of 12:12 h dark: light, the salinity of 26 ± 1 ppt, the temperature of 26 ± 2 ℃, and the DO of 5 mg L^{-1} by using continuous aeration. The pH, NH_3, NO_3, and NO_2 levels were checked regularly and were within suitable limits for shrimp farming as reported elsewhere (APHA, 2005). The values for pH were 7.70 ± 0.15, NH_3 was 0.08 ± 0.01 mg L^{-1}, NO_3 was 0.18 ± 0.02 mg L^{-1}, and NO_2 was 0.10 ± 0.01 mg L^{-1} (Boyd and Tucker, 2012). The hapa nets were cleaned frequently throughout the experiment and each pond had a water exchange of approximately 10% per day through the intake and output flow rates of the pond system.

2.5. Shrimp Tested Parameters

2.5.1. Growth Performance and Feed Utilization

To calculate the weight mass gain (WG, g), postlarvae weights (g) were determined using a digital laboratory analytical balance (Digital PX224 Ohaus Pioneer Analytical Balance, Capacity: 0.0001) at the beginning of the experiment (0.053±0.001 g, $n = 10$) and the end of the feeding trial. The average of IBW was calculated as an average between 10 PLs, repeated three times. Obtained data were used to calculate the survival rate (SR, %), specific growth rate (SGR %/day), and feed conversion ratio (FCR). Moreover, the feed efficiency ratio (FER) and protein efficiency ratio (PER) were calculated according to the following equations:

$$\text{Weight Gain}(\text{WG, g}) = \text{Final body weight}(\text{g}) - \text{Initial body weight}(\text{g}) \quad (1)$$

$$\text{Survival Rate}(\text{SR, \%}) = \frac{\text{Total final survived number of shrimp}}{\text{The initial number of shrimp}} \times 100 \quad (2)$$

$$\text{Specific Growth Rate}(\text{SGR\%}/\text{day}) = \frac{\text{Ln Final body weight} - \text{Ln Initial body weight}}{t} \times 100 \quad (3)$$

$$\text{Feed Conversion Ratio}(\text{FCR}) = \frac{\text{Total consumed feed}}{\text{WG}} \quad (4)$$

$$\text{Feed Efficiency Ratio}(\text{FER}) = \frac{\text{WG}(\text{g})}{\text{Feed intake}(\text{g})} \quad (5)$$

$$\text{Protein Efficiency Ratio}(\text{PER}) = \frac{\text{WG}(\text{g})}{\text{Protein intake}(\text{g})} \quad (6)$$

2.5.2. Body Chemical Analysis

At the end of the feeding trial, five samples were taken from each replicate to determine the chemical composition of the whole shrimp body. The shrimp were selected randomly, euthanised and blended, dried, powdered, and kept at a temperature of – 20°C for future analysis. The crude protein, crude lipid, ash, and dry matter were determined using the methods previously described by AOAC (2003).

2.5.3. Immunological Parameters

Five shrimp samples were chosen randomly from each replicate, after 24 hours of fasting, and washed, with sterile seawater for a few seconds. Shrimp were euthanized and selected organs and tissue was dissected, weighed, and frozen at – 80 ℃ until analysis. Prior to lysozyme, antioxidants, and digestive enzyme analysis, the shrimp samples were homogenized in PBS (pH 7.4), centrifuged for 20 minutes at a speed of 2000 to 3000 rpm, and the supernatant was carefully collected. For measurements of serum lysozyme activity, the Fish Lysozyme (LZM) ELISA Kits (Cat NO.:SL0050FI, SunLong Biotech Co., LTD, China) were used. The assay involved incubation of the lysozyme containing sample with *Micrococcus lysodeikticus* cells which served as substrate. The reaction was monitored by tracking the decrease in absorbance reading at a wavelength of 450 nm, following the manufacturer's instructions (Harshbarger et al., 1992) to determine the rate of substrate conversion.

To assess the antioxidant activities, the levels of serum superoxide dismutase (SOD), catalase, and lipid peroxide (Malondialdehyde, MDA) were measured using colorimetric assays. The specific kits for SOD, catalase, and MDA were purchased from Biodiagnostic Co., Egypt (Cat NO.: SD2521, CA2517, and MD2529) respectively. The determination of SOD was performed at a wavelength of 560 nm (Nishikimi et al., 1972), catalase was determined at 510 nm (Aebi, 1984), while the determination of MDA was carried out at 534 nm (Ohkawa et al., 1979). For the assays on digestive enzyme activities, the obtained homogenized tissues from the gastrointestinal (GI) tract were separated through careful centrifugation. The activities of different digestive enzymes, such as lipase and amylase, were analyzed following the instructions provided by the manufacturer. The determination of lipase and amylase was conducted using spectrophotometric assays with specific kits (Cat NO.: 281001 Spectrum, Egy. Co. Biotech., Egypt, and Cat. NO.: AY1050, Biodiagnostic Co., Egypt, respectively) at wavelengths of 580 nm (Moss et al., 1999) and 660 nm (Caraway, 1959), respectively.

2.5.4. Immune-Related Gene Expressions

At the end of the feeding trial, three equal independent samples of each five shrimp samples (whole animals) were collected, washed twice using PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.46 mM KH_2PO_4, and pH 7.4), and stored in RNA later® reagent (Sigma-Aldrich®; 1w:5 v) at – 20°C as described by Goncalves et al. (2014). The total RNA extraction and quantitative real-time PCR followed the method by Aguilera-Rivera et al. (2019). Briefly, the TRIzol reagents protocol (TRIzol©; Life Technologies) was applied to extract the total amount of RNA then the obtained extraction was quantified at 260 and 280 nm using a NanoDrop spectrophotometer (Thermo Scientific). The RT2 First Strand Kit, which includes a highly successful genomic DNA removal step before reverse transcription, was used during the RNA extraction process to prevent DNA contamination. The target estimated gene amplification efficiency was optimized for each primer set using a 10-fold dilution series of cDNA from five represntive replicates for each specimens. cDNA was produced in a 10-μL estimated volume including 4 μg of the total extracted RNA, 10 × RT buffer, 10 mM dNTP, 10× random RT primers and U reverse transcriptase (Enhanced Avian RT First Strand Synthesis; Sigma-Aldrich©). The first strand cDNA was generated at 59 °C for 50 min. Then, the designed primers studied in this experiment were designed employing NCBI tool with Primer 5.0 software (Hassan et al., 2023), including the Peroxiredoxin (*Prx*),

Table 3
Nucleotide primers used to amplify the selected genes from whiteleg shrimp with Quantitative Real-Time-PCR.

Gene	Nucleotide primers	Accession NO.	Amplification length (bp)	°C	References
Peroxiredoxin (Prx)	F: CATCTTCAAGGGCACTGCTG R: CGGCCTTCATTGTCTTGGAG	GQ995702	139	60	(**Liu et al., 2022**)
Prophenoloxidase (PPO1)	F: CCAGCAGCGTCTTCTTTACC R: GTTCAATTTCTCGCCCAGGA	AY723296	122	60	(**Wang et al., 2008**)
P53-like protein isoform delta (p53)	F: CCAAGCAGCAATGTGTCAGT R: TCAGGCTGCCACTTCTTGAT	KX827274	190	60	(**Nuñez-Hernandez et al., 2021**)
Hemocyanin subunit L5 (L5H)	F: ATGCTCATCGTTGAAACCCG R:TCGTGTTTTGAAATGACCTTGG	X82502	124	65	(**Wang et al., 2008**)
β-actin	F: ACTGGGACGACATGGAGAAG R: CAGGAATGAGGGCTGGAACA	JF288784	121	60	(Hassan et al., 2023)

Prophenoloxidase (*PPO1*), p53-like protein isoform delta (*p53*), and hemocyanin subunit L5 (*L5H*) genes (Table 3), which was conveyed into a fluorometric iQ5 thermocycler (Bio-Rad®) following the guidelines as previously described by Aguilera-Rivera et al. (2019). The gene β-actin was applied as the housekeeping gene (Wang et al., 2007). The expression level of each gene was estimated and calculated using the $^{2\text{-}\Delta\Delta}C_t$ as described by Livak and Schmittgen (2001), where C_t is the value corresponding to the number of cycles in which the fluorescence was created. Each real-time PCR reaction (including cDNA synthesis) was repeated triplicate times to approve the accuracy of the obtained results. Moreover, the qPCR values were $\log^2$ transformed to achieve normality and diminish data variability. Besides, the PCR efficiency for each sample was derived from the slope of the regression line fitted to a subset of baseline-corrected data points in the log-linear phase using LinRegPCR following the procedure reported by Ramaker et al. (2003).

2.6. Statistical Analysis

The findings of this feeding study were presented ($n = 5$) as means ± standard deviation. Before statistical analysis, Levene's test was employed to confirm the normality and homogeneity assumptions, and the results (%) were arc-sin transformed (Zar, 1984). The statistical analysis was conducted using the SPSS Statistics Software, which involved performing one-way ANOVA followed by the Duncan (1955) test at a significant level of $p \leq 0.05$. Finally, the figures were created using Graph Pad (Prism 8) Statistics Software (Swift, 1997).

Table 4
Impact of TAM® levels on growth performance and nutrient utilization indices of whiteleg shrimp (*L. vannamei*).

Items	$TAM_{0\%}$	$TAM_{1\%}$	$TAM_{2\%}$	$TAM_{3\%}$	$TAM_{4\%}$
FW	3.900 ±0.066^{c}	4.060 ±0.030^{b}	4.337 ±0.038^{a}	4.153 ±0.035^{b}	3.840 ±0.089^{c}
WG	3.850 ±0.066^{c}	4.010 ±0.030^{b}	4.287 ±0.038^{a}	4.103 ±0.035^{b}	3.787 ±0.095^{c}
SGR	3.347 ±0006 ^{cd}	3.363 ±0.021^{bc}	3.423 ±0012^{a}	3.393 ±0021^{ab}	3.327 ±021^{d}
FCR	2.183 ±0.076^{ab}	2.113 ±0.012^{b}	1.970 ±0.017^{c}	2.020 ±0.026^{c}	2.233 ±0.067^{a}
FER	0.458 ±0.016^{bc}	0.473 ±0.002^{b}	0.507 ±0.005^{a}	0.496 ±0.006^{a}	0.448 ±0.014^{c}
PER	1.270 ±0.046^{bc}	1.313 ±0.006^{b}	1.407 ±0.012^{a}	1.377 ±0.021^{a}	1.243 ±0.038^{c}
SR	79.90±0.96	79.70±0.56	80.10 ±0.76	79.80 ±0.87	79.70 ±1.26

TAM0%, TAM1%, TAM2%, TAM3%, and TAM4% are diets supplemented with 10, 20, 30, and 40 mL of TAM® per kg−1 diet. FW: final weight (g), WG: weight gain (WG, g), SGR: specific growth rate (SGR, % / day), FCR: feed conversion ratio, FER: feed efficiency ratio, PER: protein efficiency ratio, and SR: survival rate (%). Data were represented as means ± SD (n = 3). Different letters in each column indicate significant differences ($p < 0.05$). The absence of letters in each column means that there are no significant differences.

3. Results

3.1. Growth Performance and Nutrient Indices

Table 4 shows the impact of TAM®'s supplementation levels on the growth performances of whiteleg shrimp. No statistically significant differences ($p < 0.05$) were revealed in survival rates between the experimented groups. However, the results showed that shrimps fed TAM® ($TAM_{1\%}$, $TAM_{2\%}$, and $TAM_{3\%}$) exhibited significant ($p < 0.05$) higher FW, WG, SGR, FCR, FER, and PER, compared to the control group ($TAM_{0\%}$). However, compared to the control group, shrimp fed $TAM_{4\%}$ showed a significant ($p < 0.05$) decrease in SGR, FCR, FER, and PER values while no significant ($p < 0.05$) difference in the FW and WG between the $TAM_{4\%}$ group *vs*. the control group. Overall, Table 4 showed that shrimp fed the TAM_2% diet displayed the highest significant ($p < 0.05$) values of FW, WG, SGR, FER, and PEF while significantly ($p < 0.05$) lowest FCR value was revealed, compared to the other TAM groups ($TAM_{1\%}$, $TAM_{3\%}$, and TAM $_{4\%}$), as well as the control group ($TAM_{0\%}$).

3.2. Shrimp Biochemical Composition

Fig. 1 shows the biochemical composition of the shrimp diets supplemented with different TAM levels. The significantly ($p < 0.05$) highest dry matter content (%) (Fig. 1A) was observed in shrimp fed the $TAM_{4\%}$ diet, followed by $TAM_{3\%}$ and $TAM_{1\%}$, while the lowest dry matter content was observed in shrimp fed $TAM_{2\%}$ and $TAM_{0\%}$ (control group). Fig. 1B revealed that increasing the TAM inclusion level (1%, 2%, 3%, and 4%) significantly ($p < 0.05$) increased the protein content of shrimp, compared to the control group ($TAM_{0\%}$). Compared to the control group ($TAM_{0\%}$), all the TAM groups ($TAM_{1\%}$, $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$) revealed significantly ($p < 0.05$) highest lipid content (Fig. 1C). The significantly ($p < 0.05$) highest ash content (Fig. 1D) was observed by feeding the shrimp the $TAM_{0\%}$ and $TAM_{4\%}$ diets, compared to the other TAM groups.

3.3. Immunological responses

Fig. 2 shows the impact of TAM® levels on immunological responses, redox status, and digestive enzyme secretions of whiteleg shrimp. The results showed a significant difference ($p < 0.05$) in lysozyme activity and SOD of shrimp fed the control diet ($TAM_{0\%}$) *vs*. the other TAM groups ($TAM_{1\%}$, $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$).

The shrimp lysozyme activity and SOD levels (Fig. 2A and B, respectively) were found to be highest in shrimp that were fed the $TAM_{2\%}$ diet. This was followed by shrimp fed the $TAM_{3\%}$, $TAM_{4\%}$, and $TAM_{1\%}$ diets, while the lowest activity levels were observed in shrimp fed the control diet ($TAM_{0\%}$). The MDA values, which indicate lipid peroxidation, were significantly lower ($p < 0.05$) in shrimp-fed diets supplemented with TAM ($TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$) compared to the control group and $TAM_{1\%}$ (Fig. 2C). The shrimp fed the $TAM_{2\%}$ diet exhibited the highest catalase CAT value (Fig. 2D) compared to the other

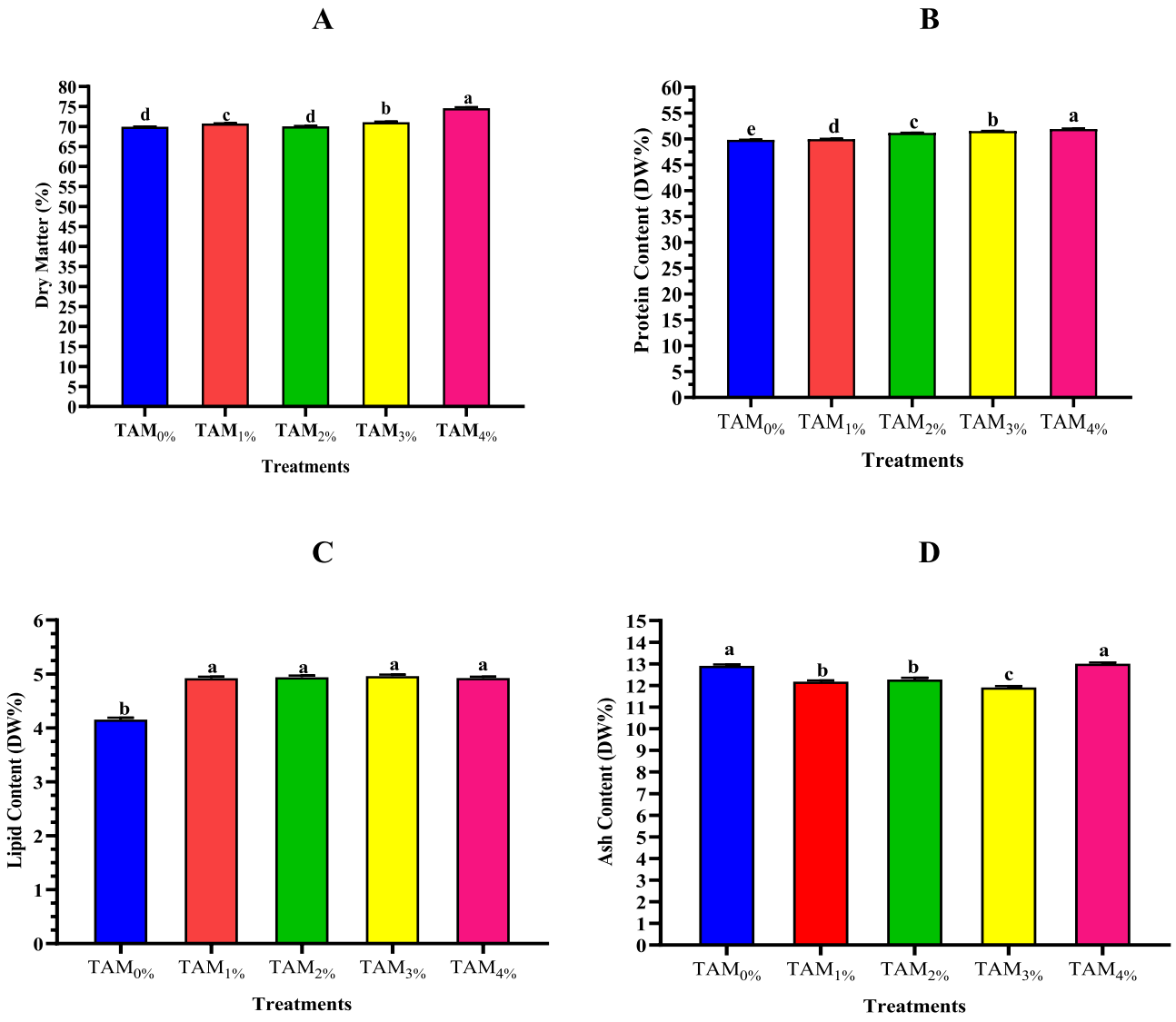

Fig. 1. Effect of TAM® diet supplementation on whole-body biochemical composition of whiteleg shrimp of dry matter (A), protein (B), lipid (C), and ash (D) content. $TAM_{0\%}$, $TAM_{1\%}$, $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$ are diets supplemented with 10, 20, 30, and 40 mL of TAM® per kg^{-1} diet. Data were represented as means ± SD. Different letters in each column indicate significant differences ($p < 0.05$).

experimental groups ($TAM_{0\%}$, $TAM_{1\%}$, $TAM_{3\%}$, and $TAM_{4\%}$). When examining the digestive enzyme activities (Fig. 2E and F), the highest activities of lipase and amylase were observed in shrimp fed the $TAM_{2\%}$ diet, followed by those fed the $TAM_{3\%}$, $TAM_{4\%}$, $TAM_{1\%}$, and the control diet $TAM_{0\%}$.

3.4. Immune-Related Gene Expressions

Fig. 3 displayed the mRNA transcription of the *Prx*, *PPO1*, *P53*, and *L5H* genes in shrimp-fed diets supplemented with several levels of TAM, and increased TAM levels significantly increased *Prx* gene transcription (up to 40 mL kg^{-1} diet). Compared to other groups, $TAM_{3\%}$ displayed the greatest *Prx* gene transcription, followed by $AM_{1\%}$ and $TAM_{0\%}$ (Fig. 3A). Moreover, the $TAM_{1\%}$ group considerably increased *PPO1* gene transcription, but the $TAM_{0\%}$, $TAM_{2\%}$, and $AM_{3\%}$ groups maintained the same *PPO1* gene transcription tendencies (Fig. 3B). In contrast, the $TAM_{2\%}$ revealed significantly ($p < 0.05$) higher *P53* gene transcription (Fig. 3C) compared to the control group ($TAM_{0\%}$) or the other inclusion levels of TAM ($TAM_{1\%}$, $TAM_{3\%}$, and $TAM_{4\%}$). Finally, shrimp diets supplemented with TAM ($TAM_{1\%}$, $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$) significantly ($p < 0.05$) improved *L5H* gene transcription (Fig. 3D) *vs*. the control group. By feeding the $TAM_{1\%}$ diet the highest levels of *L5H* gene expression were revealed, followed by the $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$ diets, respectively.

4. Discussion

Worldwide, whiteleg shrimp farming has been rapidly evolving, leading to increases in demand for specialized feed additives to be used in bespoke aquafeed formulations. This trend has created a significant opportunity for the aquaculture industry to use seaweed extract (Mabrouk et al., 2022). Seaweeds and/or their extracts have a greater potential for commercial aquaculture utilization compared to other aquatic plant or even invertebrate organisms (Metwally et al., 2020). In agreement with the current findings, (Ashour et al. 2020b; **2021**) reported that inclusion levels of TAM®, within specific ranges in diets (5, 10, 15, and 20 g kg^{-1} diet) or administered in the water culture containment (50, 100, 150, and 200 mL m^{-3} of culture water), significantly improved the growth performances, feed utilization, and whole-body composition of Nile tilapia. These improvements may be attributed to the phytochemical potential of TAM®, which was previously reported as a growth enhancer for fish and plants (Mekki, 2015;

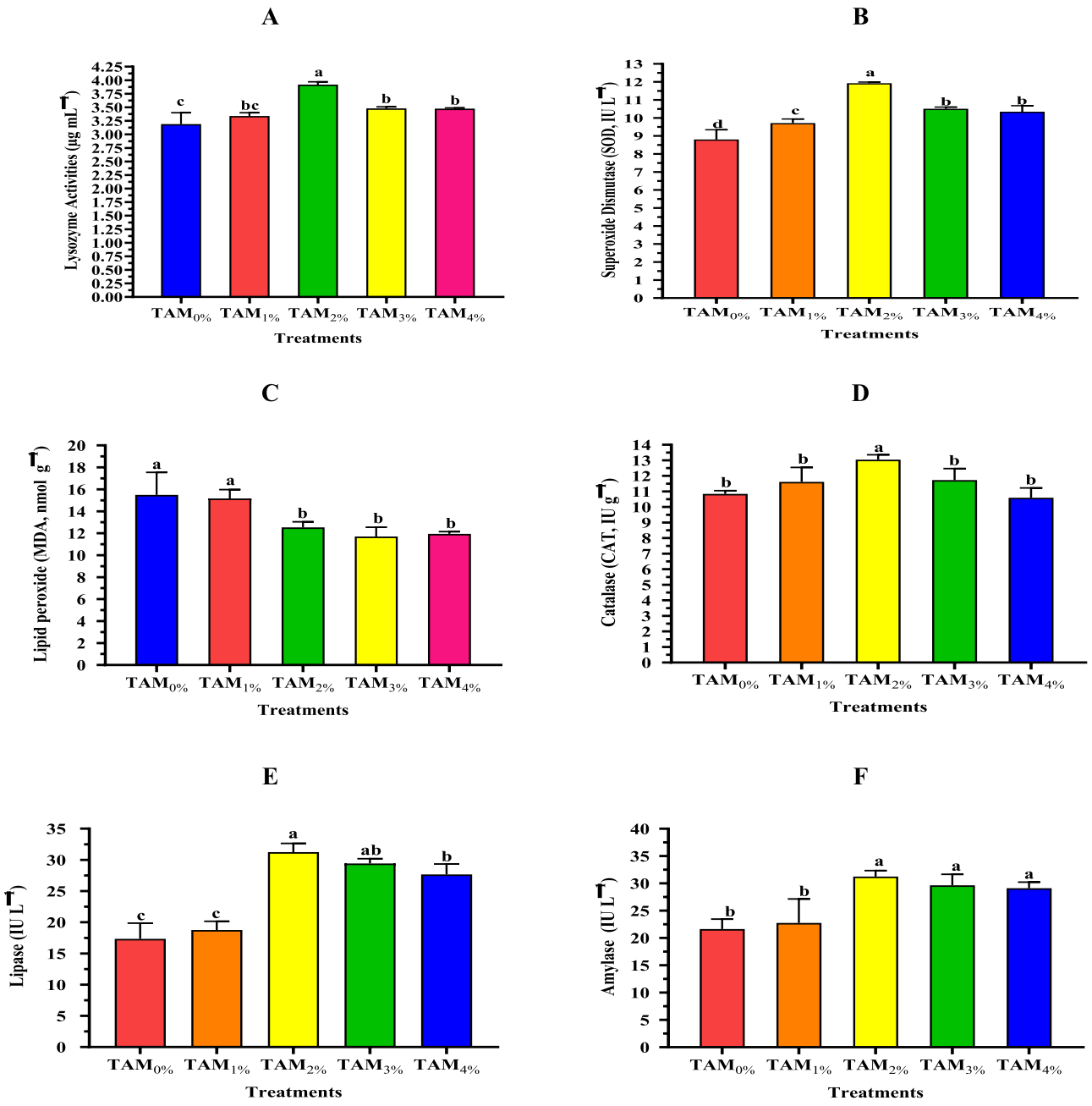

Fig. 2. Effect of TAM® diet supplementation on immunological indicators of whiteleg shrimp of (A) lysozyme activities (µg mL^{-1}), (B) superoxide dismutase (SOD, IU L^{-1}), (C) lipid peroxide (MDA, nmol g^{-1}), (D) catalase (CAT, U g^{-1}), (E) lipase (U L^{-1}), and (F) amylase (IU L^{-1}). $TAM_{0\%}$, $TAM_{1\%}$, $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$ are diets supplemented with 10, 20, 30, and 40 mL of TAM® per kg^{-1} diet. Data were represented as means ± SD. Different letters in each column indicate significant differences ($p < 0.0$).

Laane, 2018; Ashour et al., 2020b). As previously reported by Ashour et al. (2020b), the GC-Mass analysis of TAM® showed nine important bioactive compounds categorized into seven groups. Seven of these bioactive materials revealed growth-promoting and immunity-enhancing activities, as well as antioxidant and antimicrobial properties. These compounds include oleic acid, rhodopin, phytol, nonadecane, and three fatty acid compounds (Table 2). Moreover, two additional novel phytochemical compounds were discovered in seaweed extract (Ashour et al., 2020b), Silaspiro[4.4]nona-1,3,6,8-tetraene,3,8-bis(diethylboryl)-2,7-diethyl-1,4,6,9-tetraphenyl-, and milbemycin-oxime.

Numerous studies concluded that several seaweed extracts have comprehensive positive potential applications as feed additives for different shrimp species such as whiteleg shrimp (Cantelli et al., 2019; Lee et al., 2020; Abbas et al., 2023), *Penaeus monodon* (the Black tiger shrimp) (Immanuel et al., 2010; Kanjana et al., 2011; Immanuel et al., 2012; AftabUddin et al., 2021), and *Fenneropenaeus merguiensis* (the Banana shrimp). Liu et al. (2020), Liu et al. (2020) examined how the growth, feed utilization, biochemical analysis, microbial abundance, and gene expression related to growth, stress, and immunity of whiteleg shrimp were modulated by the addition of different amounts (1, 2, and 3 g kg^{-1} of diet) of polysaccharides extracted from brown seaweed *S. dentifolium*. Abbas et al. (2023) reported that adding polysaccharides based on seaweed to the diets of shrimp led to significant improvements in WG and SR compared to control fed shrimp. The whole-body biochemical composition of the shrimp and microbial abundance (count of heterotrophic bacteria and *Vibrio* spp.) also showed significant differences among the diets with added polysaccharides compared to the

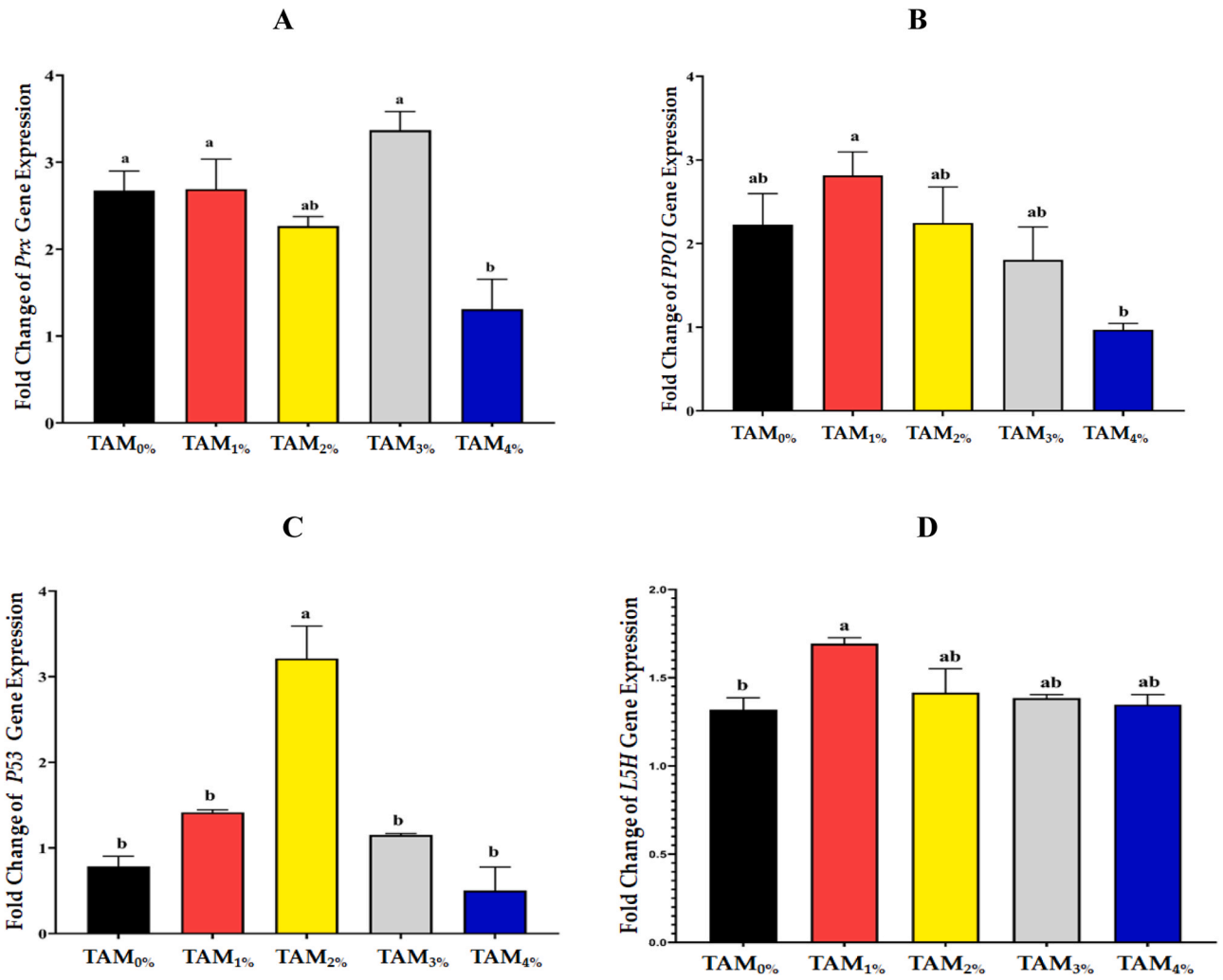

Fig. 3. Effect of TAM® diet supplementation on mRNA expression folds changes of (A) the Peroxiredoxin (Prx), (B) Prophenoloxidase (PPO1), (C) p53-like protein isoform delta (P53), and (D) Hemocyanin subunit L5 (L5H) genes of whiteleg shrimp. $TAM_{0\%}$, $TAM_{1\%}$, $TAM_{2\%}$, $TAM_{3\%}$, and $TAM_{4\%}$ are diets supplemented with 10, 20, 30, and 40 mL of TAM® per kg^{-1} diet. Data were represented as means ± SD. Different letters in each column indicate significant differences ($p < 0.0$).

control group. Moreover, the expression of growth-related genes (*IGF-I* and *IGF-II*), immune-related genes (*β-Bgp, ProPO, Lys*, and *Crustin*), and stress associated genes (*SOD* and *GPx*) in the muscle tissue of whiteleg shrimp increased with the dietary supplementation of seaweed polysaccharide extract. However, the study concluded that an inclusion of 2 g kg^{-1} polysaccharide as a dietary additive led to the best results, improving WG and SR, while the incorporation of 3 g kg^{-1} reduced the number of harmful microbes such as *Vibrio* spp., and enhanced the shrimp's growth, immunity, and stress-related gene expression portfolio (Abbas et al., 2023). According to the study by Lee et al. (2020), the hot-water extract of brown macroalga *Sargassum horneri* had a positive response on the growth, innate immunity, and immune-related gene expressions of whiteleg shrimp, and the authors recommended that adding different levels of seaweed hot-water extract (0.25, 0.5%, and 1%) to the shrimp's diet, the ideal inclusion level was 0.5% (5 g kg^{-1}). The stimulation of the immune system through diet has been widely investigated in aquaculture as a means of increasing shrimp resistance to infectious diseases (Abbas et al., 2023).

AftabUddin et al. (2021) studied, for 45 days, the impact of the methanolic and ethanolic extracts (0.25% and 5% of kg^{-1} diet) of two brown seaweed species (*Sargassum ilicifolium* and *Padina tetrastromatica*) on survival, growth performance, antibacterial properties, and immune responses of tiger shrimp (*Penaeus monodon*) challenged with *Vibrio parahaemolyticus,* and the authors concluded that the most favorable growth performance and survival rates of juvenile tiger shrimps were achieved when administered *P. etrastromatica* extract at a dose of 5 g kg^{-1}. The highest antibacterial activity against *V. parahaemolyticus* was observed in the methanol extract of both *P. tetrastromatica* and *S. ilicifolium*. Furthermore, administering a combination of methanol and ethanol extract of *P. tetrastromatica* at a dose of 5 g kg^{-1} resulted in improved growth performance and immune capacity of tiger shrimp.

Immanuel et al. (2010) investigated, for 20 days, the impact of a hot water extract of *Sargassum duplicatum* and *Sargassum wightii* (brown seaweed) on the growth performances and WSSV resistance in tiger shrimp postlarvae (PL15–35). The results revealed that the treated groups showed significantly higher WG and SGR than the control group, without seaweed extract supplementation. Moreover, the control group showed 100% mortality within the 8th day, whereas the highest inclusion rate of seaweed extract (750 mg L^{-1}) yielded the lowest mortality (54–79%). These findings provide evidence that supports the consideration of hot water extracts of *S. duplicatum* and *S. wightii*, as potential alternatives to antibiotics for managing WSSV disease.

In another study by Immanuel et al. (2012), the impact of fucoidan (polysaccharide extracted from *S. wightii*) dietary supplementation, for 45 days, on the immune activities of *Penaeus monodon* challenged with WSSV was studied and all recorded immune activities (such as prophenoloxidase activity, respiratory burst activity, superoxide dismutase activity and phagocytic activity) showed significantly elevated values

than the control group. The same findings were also confirmed by Kanjana et al. (2011) when using the solvent extracts of *Gracilaria fisheri* (red seaweed) as dietary supplementation for tiger shrimp (Kanjana et al., 2011). Liu et al. (2020) conducted a study to examine how different levels of polysaccharides extracted from *Enteromorpha* sp. (green seaweed) supplemented with the diet of Banana shrimp (*F. merguiensis*) affected growth, immunity, and intestinal function, and the study revealed that inclusion of 1 g of polysaccharides per kg diet resulted in significant improvements in growth performance, enhanced nonspecific immunity, and modulated the intestinal function of Banana shrimp (Liu et al., 2020).

Niu et al. (2019) reported that the intestinal microbiota of whiteleg shrimp was modulated by the administration of dietary seaweed *Saccharina japonica*. Mansour et al. (2022b) reported that dietary supplementation with natural astaxanthin extracted from *A. platensis* NIOF17/003 significantly improved the growth, gut bacterial populations, and genes expression of immune and antioxidant-related genes of whiteleg shrimp. According to the study by Chien and Shiau (2005), astaxanthins derived from several algae strains (*H. pluvialis* and *A. pacifica*) significantly enhance the growth and resistance to hypoxia stress in the juvenile of *Marsupenaeus japonicus* (Kuruma shrimp). The same findings were reported by Zhang et al. (2021) with *Exopalaemon carinicauda* (the Ridgetail white prawn). The results of these studies are consistent with the findings of the present study, demonstrating that seaweed extracts have the potential as a feed additive in shrimp aquaculture. The results of the present study indicate that TAM® extract enhanced growth performances, induced and supported immunity, and promoted immune-related gene expression in whiteleg shrimp. Based on these observations, the current study concludes that TAM® represents a promising, eco-friendly, and sustainable option for use in shrimp aquaculture.

5. Conclusions

The commercial seaweed extract (TAM®) was previously reported as a desirable feed additive and water conditioner for Nile tilapia (*O. niloticus*). Based on the findings of the current study, it is concluded that, in addition, TAM® is a viable source of seaweed phytochemicals with high potential applicability in the aquafeed-additive industry for the whiteleg shrimp. The optimal inclusion rate of TAM® as an aquafeed-additive for *L. vannamei* was a subject of inquiry in this study. The results of the feeding trial revealed that the shrimp in the group receiving 2% TAM® (20 mL of TAM® kg^{-1} diet) exhibited significant improvements in growth and feed utilization performance (FW, SGR, FCR, PER, and FER), whole-body composition (protein and lipid content), immune responses (lysozyme activities, SOD, catalase, lipase, and amylase), and immune-related gene expressions (*p53*) of the shrimp. In conclusion, this study demonstrates that TAM® is a sustainable, eco-friendly, and promising option for utilization in the aquafeed-additive industry in meeting the sustainability agenda for resilient shrimp production.

Ethical approval

The experiments presented in this manuscript were subjected to the general protocol standards for the Institutional Animal Care and Use Committee of the National Institute of Oceanography and Fisheries (NIOF).

CRediT authorship contribution statement

Ahmed I. A. Mansour: Methodology, Investigation. **Elsayed M. Younis:** Validation, Software, Resources. **Ehab El-Haroun:** Writing – review & editing, Visualization, Supervision, Resources, Investigation. **Mohammed A.E. Naiel:** Software, Methodology, Formal analysis. **Ahmed F. Abdelhamid:** Resources, Methodology, Investigation, Data curation. **Mohamed M. Mabrouk:** Methodology, Investigation, Conceptualization. **Ahmed A. A. El-Bahlol:** Software. **Mohamed Ashour:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Einar Ringø:** Writing – original draft, Visualization, Validation, Supervision. **Ahmed Said Al-Souti:** Writing – original draft, Software. **Mohamed A. Elokaby:** Writing – original draft. **Mohammad S. Abu Husein:** Validation, Formal analysis. **Abeer El-Saharty:** Writing – review & editing. **Abdelwahab A. Abdelwarith:** Writing – original draft, Funding acquisition, Formal analysis. **Marwa F. AbdEl Kader:** Visualization, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

True-Algae-Max (TAM®) was produced as an output product (know-how) of the research project funded by the Academy of Scientific Research and Technology (ASRT), Egypt, under the title (Prototype of Sustainable Marine Integrated Aquaculture Farm for the Production of Seafood, Valuable Bioproducts, and Biodiesel, Project ID: 1429). The authors appreciated The Academy of Scientific Research and Technology (ASRT), Egypt (SIMAF-Project Project ID: 1429), and Researchers Supporting project number (RSP2024R36), King Saud University, Riyadh, Saudi Arabia, for funding and supporting this work.

Patents

Seaweed extract (TrueAlgaeMax, TAM®) is a patent (Ashour, 2019) submitted at the Egyptian Patents Office (EPO), Academy of Scientific Research and Technology (submission No.: 2046/2019).

References

Abbas, E.M., Ali, F., Desouky, S., Ashour, M.G., El-Shafei, M., Maaty, A., Sharawy, M.M., Z.Z, 2020. Novel comprehensive molecular and ecological study introducing coastal mud shrimp (*Solenocera crassicornis*) recorded at the gulf of suez, egypt. J. Mar. Sci. Eng. 9, 9.

Abbas, E.M., Al-Souti, A.S., Sharawy, Z.Z., El-Haroun, E., Ashour, M., 2023. Impact of dietary administration of seaweed polysaccharide on growth, microbial abundance, and growth and immune-related genes expression of the pacific whiteleg shrimp (*Litopenaeus vannamei*). Life 13, 344.

Abisha, R., Krishnani, K.K., Sukhdhane, K., Verma, A., Brahmane, M., Chadha, N., 2022. Sustainable development of climate-resilient aquaculture and culture-based fisheries through adaptation of abiotic stresses: A review. J. Water Clim. Change.

Abo-Taleb, H.A., Ashour, M., Elokaby, M.A., Mabrouk, M.M., El-feky, M.M.M., Abdelzaher, O.F., Gaber, A., Alsanie, W.F., Mansour, A.T., 2021. Effect of a new feed *Daphnia magna* (straus, 1820), as a fish meal substitute on growth, feed utilization, histological status, and economic revenue of grey mullet, *Mugil cephalus* (linnaeus 1758). Sustainability 13, 7093.

Abo-Taleb, H.A., Ashour, M., El-Shafei, A., Alataway, A., Maaty, M.M., 2020a. Biodiversity of calanoida copepoda in different habitats of the north-western red sea (hurghada shelf). Water 12, 656.

Abo-Taleb, H.A., F Zeina, A., Ashour, M., M Mabrouk, M., E Sallam, A., MM El-feky, M., 2020b. Isolation and cultivation of the freshwater amphipod *Gammarus pulex* (linnaeus, 1758), with an evaluation of its chemical and nutritional content. Egypt. J. Aquat. Biol. Fish. 24, 69–82.

Aebi, H., 1984. [13] catalase in vitro. Methods in enzymology. Elsevier, pp. 121–126.

AftabUddin, S., Siddique, M.A.M., Habib, A., Akter, S., Hossen, S., Tanchangya, P., Abdullah Al, M., 2021. Effects of seaweeds extract on growth, survival, antibacterial activities, and immune responses of *Penaeus monodon* against *Vibrio parahaemolyticus*. Ital. J. Anim. Sci. 20, 243–255.

Aguilera-Rivera, D., Rodríguez-Fuentes, G., Escalante-Herrera, K.S., Guerra-Castro, E., Prieto-Davó, A., Gaxiola, G., 2019. Differential expression of immune-related genes

in pacific white shrimp, *Litopenaeus vannamei*, previously reared in biofloc and challenged with *vibrio harveyi*. Aquac. Res. 50, 2039–2046.
Ahmed, N., Thompson, S., Glaser, M., 2019. Global aquaculture productivity, environmental sustainability, and climate change adaptability. Environ. Manag. 63, 159–172.
Ahmed, N., Turchini, G.M., 2021. Recirculating aquaculture systems (ras): Environmental solution and climate change adaptation. J. Clean. Prod. 297, 126604.
Alam, M.Z., Braun, G., Norrie, J., Hodges, D.M., 2013. Effect of ascophyllum extract application on plant growth, fruit yield and soil microbial communities of strawberry. Can. J. Plant Sci. 93, 23–36.
Alprol, A.E., Heneash, A.M.M., Ashour, M., Abualnaja, K.M., Alhashmialameer, D., Mansour, A.T., Sharawy, Z.Z., Abu-Saied, M.A., Abomohra, A.E., 2021. Potential applications of *arthrospira platensis* lipid-free biomass in bioremediation of organic dye from industrial textile effluents and its influence on marine rotifer (*Brachionus plicatilis*). Mater. (Basel) 14.
Anh, N.T.N., Shayo, F.A., Nevejan, N., Van Hoa, N., 2021. Effects of stocking densities and feeding rates on water quality, feed efficiency, and performance of white leg shrimp Litopenaeus vannamei in an integrated system with sea grape Caulerpa lentillifera. J. Appl. Phycol. 33, 3331–3345.
AOAC, 2003. Official methods of analysis of the association of official analytical chemists. The Association.
APHA, 2005. Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA.
Ashour, M., 2019. Bioactive compounds extracted from marine algae improve the growth and immunity of plants, fish and marine crustaceans. Egyptian patent office, egypt (under investigation), Academy of Scientific Research and Technology (ASRT), Egypt.
Ashour, M., Alprol, A.E., Heneash, A.M.M., Saleh, H., Abualnaja, K.M., Alhashmialameer, D., Mansour, A.T., 2021a. Ammonia bioremediation from aquaculture wastewater effluents using *arthrospira platensis* niof17/003: Impact of biodiesel residue and potential of ammonia-loaded biomass as rotifer feed. Mater. (Basel) 14, 5460.
Ashour, M., Al-Souti, A.S., Hassan, S.M., Ammar, G.A.G., Goda, A., El-Shenody, R., Abomohra, A., El-Haroun, E., Elshobary, M.E., 2023. Commercial seaweed liquid extract as strawberry biostimulants and bioethanol production. Life 13, 85.
Ashour, M., El-Shafei, A.A., Khairy, H.M., Abd-Elkader, D.Y., Mattar, M.A., Alataway, A., Hassan, S.M., 2020a. Effect of *Pterocladia capillacea* seaweed extracts on growth parameters and biochemical constituents of jew's mallow. Agronomy 10, 420.
Ashour, M., Mabrouk, M.M., Abo-Taleb, H.A., Sharawy, Z.Z., Ayoub, H.F., Van Doan, H., Davies, S.J., El-Haroun, E., Goda, A.M.S.A., 2021b. A liquid seaweed extract (tam®) improves aqueous rearing environment, diversity of zooplankton community, whilst enhancing growth and immune response of nile tilapia, *Oreochromis niloticus,* challenged by *Aeromonas hydrophila.* Aquaculture 543, 736915.
Ashour, M., Mabrouk, M.M., Ayoub, H.F., El-Feky, M.M.M.M., Z, S.Z, Hoseinifar, S.H., Rossi, W., Van Doan, H., El-Haroun, E., Goda, A.M.A.S., 2020b. Effect of dietary seaweed extract supplementation on growth, feed utilization, hematological indices, and non-specific immunity of nile tilapia, *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. J. Appl. Phycol. 32, 3467–3479.
Boyd, C.E., Tucker, C.S., 2012. Pond aquaculture water quality management. Springer Science & Business Media.
Cantelli, L., Goncalves, P., Guertler, C., Kayser, M., Pilotto, M.R., Barracco, M.A., Perazzolo, L.M., 2019. Dietary supplementation with sulfated polysaccharides from gracilaria birdiae promotes a delayed immunostimulation in marine shrimp challenged by the white spot syndrome virus. Aquac. Int. 27, 349–367.
Caraway, W., 1959. A-amylase colorimetric method. Ame. J. Clin. Pathol. 32, 97–99.
Chien, Y.-H., Shiau, W.-C., 2005. The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth, and low dissolved oxygen stress resistance of kuruma prawn, *Marsupenaeus japonicus* bate. J. Exp. Mar. Biol. Ecol. 318, 201–211.
Duncan, D.B., 1955. Multiple range and multiple f tests. biometrics 11, 1–42.
El Nemr, A., Khaled, A., Moneer, A.A., El Sikaily, A., 2012. Risk probability due to heavy metals in bivalve from egyptian mediterranean coast. Egypt. J. Aquat. Res. 38, 67–75.
El-Said, G.F., El-Sikaily, A., 2013. Chemical composition of some seaweed from mediterranean sea coast, egypt. Environ. Monit. Assess. 185, 6089–6099.
El-Sayed, A.F.M., 2021. Use of biofloc technology in shrimp aquaculture: A comprehensive review, with emphasis on the last decade. Rev. Aquac. 13, 676–705.
Elshobary, M.E., El-Shenody, R., Abomohra, A.E.-f, 2020. Sequential biofuel production from seaweeds enhances the energy recovery: A case study for biodiesel and bioethanol production. International Journal of Energy Research.
Emerenciano, M.G., Rombenso, A.N., Vieira, Fd.N., Martins, M.A., Coman, G.J., Truong, H.H., Noble, T.H., Simon, C.J., 2022. Intensification of penaeid shrimp culture: An applied review of advances in production systems, nutrition and breeding. Animals 12, 236.
Fawzy, S., Wang, W., Wu, M., Yi, G., Huang, X., 2022. Effects of dietary different canthaxanthin levels on growth performance, antioxidant capacity, biochemical and immune-physiological parameters of white shrimp (Litopenaeus vannamei). Aquaculture 556, 738276.
Gillett, R., 2008. Global study of shrimp fisheries. FAO Fish. Tech. Pap. 475, 25–29.
Goncalves, P., Guertler, C., Bachère, E., de Souza, C.R., Rosa, R.D., Perazzolo, L.M., 2014. Molecular signatures at imminent death: Hemocyte gene expression profiling of shrimp succumbing to viral and fungal infections. Dev. Comp. Immunol. 42, 294–301.
Harshbarger, J.C., Spero, P.M., Wolcott, N.M., 1992. Neoplasms in wild fish from the marine ecosystem. Pathobiol. Mar. Estuar. Org. 2, 157.
Hassan, S.A., Sharawy, Z.Z., Hemeda, S.A., El Nahas, A.F., Abbas, E.M., Khalil, H.S., Verdegem, M., 2023. Sugarcane bagasse improved growth performance, digestive enzyme activity, microbial dynamics, and mrna transcripts of immune-, growth, and antioxidant-related genes of *Litopenaeus vannamei* in a zero-water exchange system. Aquac. Rep. 33, 101788.
Hassan, S., Shobier, A., 2018. Gc/ms identification and applications of bioactive seaweed extracts from mediterranean coast of egypt. Egypt. J. Aquat. Biol. Fish. 22, 1–21.
Hu, B., 2022. Effects of pelleted and extruded diets of different protein levels on growth, digestive enzyme activity, antioxidant capacity, nonspecific immunity, and ammonia-n stress tolerance of postlarval pacific white shrimp. North Am. J. Aquac. 84, 239–248.
Iber, B.T., Kasan, N.A., 2021. Recent advances in shrimp aquaculture wastewater management. Heliyon 7, e08283.
Immanuel, G., Sivagnanavelmurugan, M., Balasubramanian, V., Palavesam, A., 2010. Effect of hot water extracts of brown seaweeds sargassum spp. On growth and resistance to white spot syndrome virus in shrimp Penaeus monodon postlarvae. Aquac. Res. 41, e545–e553.
Immanuel, G., Sivagnanavelmurugan, M., Marudhupandi, T., Radhakrishnan, S., Palavesam, A., 2012. The effect of fucoidan from brown seaweed sargassum wightii on wssv resistance and immune activity in shrimp Penaeus monodon (fab). Fish. Shellfish Immunol. 32, 551–564.
Jobling, M., 1983. A short review and critique of methodology used in fish growth and nutrition studies. J. Fish. Biol. 23, 685–703.
Kanjana, K., Radtanatip, T., Asuvapongpatana, S., Withyachumnarnkul, B., Wongprasert, K., 2011. Solvent extracts of the red seaweed gracilaria fisheri prevent vibrio harveyi infections in the black tiger shrimp Penaeus monodon. Fish. Shellfish Immunol. 30, 389–396.
Khaled, A., Hessein, A., Abdel-Halim, A.M., Morsy, F.M., 2014. Distribution of heavy metals in seaweeds collected along marsa-matrouh beaches, egyptian mediterranean sea. Egypt. J. Aquat. Res. 40, 363–371.
Laane, H.-M., 2018. The effects of foliar sprays with different silicon compounds. Plants 7, 45.
Lee, P.-T., Tran, H.T.Q., Huang, H.-T., Nan, F.-H., Lee, M.-C., 2020. Sargassum horneri extracts stimulate innate immunity, enhance growth performance, and upregulate immune genes in the white shrimp Litopenaeus vannamei. Fish. Shellfish Immunol. 102, 276–285.
Li, E., Xu, C., Wang, X., Wang, S., Zhao, Q., Zhang, M., Qin, J.G., Chen, L., 2018. Gut microbiota and its modulation for healthy farming of pacific white shrimp Litopenaeus vannamei. Rev. Fish. Sci. Aquac. 26, 381–399.
Liu, W.-C., Zhou, S.-H., Balasubramanian, B., Zeng, F.-Y., Sun, C.-B., Pang, H.-Y., 2020. Dietary seaweed (*enteromorpha*) polysaccharides improves growth performance involved in regulation of immune responses, intestinal morphology and microbial community in banana shrimp *Fenneropenaeus merguiensis*. Fish. Shellfish Immunol. 104, 202–212.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2− δδct method. methods 25, 402–408.
Lukwambe, B., Nicholaus, R., Zhang, D., Yang, W., Zhu, J., Zheng, Z., 2019. Successional changes of microalgae community in response to commercial probiotics in the intensive shrimp (Litopenaeus vannamei boone) culture systems. Aquaculture 511, 734257.
Mabrouk, M.M., Ashour, M., Labena, A., Zaki, M.A.A., Abdelhamid, A.F., Gewaily, M.S., Dawood, M.A.O., Abualnaja, K.M., Ayoub, H.F., 2022. Nanoparticles of *arthrospira platensis* improves growth, antioxidative and immunological responses of nile tilapia (*Oreochromis niloticus*) and its resistance to *Aeromonas hydrophila*. Aquac. Res. 53, 125–135.
Magouz, F.I., Essa, M.A., Matter, M., Mansour, T.A., Alkafafy, M., Ashour, M., 2021. Population dynamics, fecundity and fatty acid composition of *Oithona nana* (*cyclopoida, copepoda*), fed on different diets. Anim. (Basel) 11.
Mansour, A.T., Alprol, A.E., Abualnaja, K.M., El-Beltagi, H.S., Ramadan, K.M.A., Ashour, M., 2022a. The using of nanoparticles of microalgae in remediation of toxic dye from industrial wastewater: Kinetic and isotherm studies. Mater. (Basel) 15.
Mansour, A.T., Ashour, M., Abbas, E.M., Alsaqufi, A.S., Kelany, M.S., El-Sawy, M.A., Sharawy, Z.Z., 2022b. Growth performance, immune-related and antioxidant genes expression, and gut bacterial abundance of pacific white leg shrimp, *Litopenaeus vannamei,* dietary supplemented with natural astaxanthin. Front. Physiol. 13.
Mekki, B.E.-D., 2015. Effect of boron foliar application on yield and quality of some sunflower (Helianthus annuus l.) cultivars. J. Agric. Sci. Technol. 5 (5), 309–316.
Metwally, A.S., El-Naggar, H.A., El-Damhougy, K.A., Bashar, M.A.E., Ashour, M., Abo-Taleb, H.A.H., 2020. Gc-ms analysis of bioactive components in six different crude extracts from the soft coral (*sinularia maxim*) collected from ras mohamed, aqaba gulf, red sea, egypt. Egypt. J. Aquat. Biol. Fish. 24, 425–434.
Moss, D.M., Rudis, M., Henderson, S.O., 1999. Cross-sensitivity and the anticonvulsant hypersensitivity syndrome. J. Emerg. Med. 17, 503–506.
Nishikimi, M., Rao, N.A., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849–854.
Niu, J., Xie, J.-J., Guo, T.-Y., Fang, H.-H., Zhang, Y.-M., Liao, S.-Y., Xie, S.-W., Liu, Y.-J., Tian, L.-X., 2019. Comparison and evaluation of four species of macro-algaes as dietary ingredients in *Litopenaeus vannamei* under normal rearing and wssv challenge conditions: Effect on growth, immune response, and intestinal microbiota. Front. Physiol. 9, 1880.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Osman, M.E., Abo-shady, A.M., Elshobary, M.E., 2013. Production and characterization of antimicrobial active substance from some macroalgae collected from abu-qir bay (alexandria) egypt. Afr. J. Biotechnol. 12, 6847–6858.

Parađiković, N., Teklić, T., Zeljković, S., Lisjak, M., Špoljarević, M., 2019. Biostimulants research in some horticultural plant species—a review. Food Energy Secur. 8, e00162.
Ramaker, H.-J., van Sprang, E.N., Westerhuis, J.A., Smilde, A.K., 2003. Dynamic time warping of spectroscopic batch data. Anal. Chim. Acta 498, 133–153.
Sharawy, Z.Z., Ashour, M., Labena, A., A.S, A, T, M.A, Abbas, E., 2022. Effects of dietary *arthrospira platensis* nanoparticles on growth performance, feed utilization, and growth-related gene expression of pacific white shrimp. *litopenaeus vannamei*. Aquac. 551, 737905.
Swift, M.L., 1997. Graphpad prism, data analysis, and scientific graphing. J. Chem. Inf. Comput. Sci. 37, 411–412.
Torres, M., Kraan, S., Domínguez, H., 2019. Seaweed biorefinery. Rev. Environ. Sci. Bio/Technol. 18, 335–388.
Wang, Y.-C., Chang, P.-S., Chen, H.-Y., 2007. Tissue expressions of nine genes important to immune defence of the pacific white shrimp Litopenaeus vannamei. Fish. Shellfish Immunol. 23, 1161–1177.
Zar, J.H., 1984. Statistical significance of mutation frequencies, and the power of statistical testing, using the poisson distribution. Biom. J. 26, 83–88.
Zhang, S., Chang, Z., Wang, W., Zheng, Y., Li, J., 2022. Dietary supplement of microalgal astaxanthin extraction improved shell pigmentation and nutritional value of Litopenaeus vannamei in an indoor industrial aquaculture system. Aquac. Nutr. 2022.
Zhang, C., Jin, Y., Yu, Y., Xiang, J., Li, F., 2021. Effects of natural astaxanthin from microalgae and chemically synthetic astaxanthin supplementation on two different varieties of the ridgetail white prawn (*Exopalaemon carinicauda*). Algal Res. 57, 102347.