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The potential role of marine macroalgae *Padina pavonica* on growth performance, histological status, and resistance of the rabbitfish *Siganus rivulatus* to *Pseudomonas anguilliseptica* bacteria

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ABSTRACT

Macroalgae, including *Padina pavonica* (PP) has recently gained more attention due to their high contents of bioactive components that increase their value as a natural feed additive resource in practical aquaculture. The current study investigated the effect of marine macroalga *P. pavonica* as a natural dietary feed additive on growth, histological status, and resistance of rabbitfish *Siganus rivulatus* fry against *Pseudomonas anguilliseptica* bacteria. Four diets were formulated to contain 0 (control T0), 50 (T1), 75 (T2), and 100 (T3) g PP/kg diet. Rabbitfish fry specimens with an average weight of 1.10±0.06 were divided into four treatments in triplicates. Fish samples were fed experimental diets to apparent satiation three times a day for eight weeks. After the feeding trial, fish were challenged with pathogenic bacteria (*P. anguilliseptica*) infection. The result revealed that growth performance, feed utilization, and survival (%) significantly improved with increasing PP rate in all experimental fish diets compared to the control. Upon increasing dietary PP levels, total body protein content increased. The opposite trend was observed for total body lipid content. Fish fed on diets containing PP showed protective protection against *P. anguilliseptica* bacterial infection and recorded a decrease in mortality to 22.5% in a fish-fed diet containing 10 g PP/kg, compared to the control group (72.5%). No histopathological changes were detected in the intestine, liver, and spleen organs in either the control group or the group treated with PP. The results of the current study indicate that *S. rivulatus* fry-fed diets containing at least 10g/kg of macroalgae, *P. pavonica* for eight weeks enhanced growth performance, diet utilization efficiency, and histopathological indices of the fish intestine, liver, and spleen.

1. INTRODUCTION

In many countries, macroalgae are well recognized as a primary food source, with an increasing attention as a precious food source **(MacArtain *et al.*, 2007)**. Worldwide, more than 221 macroalgal species have been reported for commercial use **(Lindsey Zemke-White & Ohno, 1999)**. The critical commercial utilization of algae as human

food, feed additive for animals, fertilizer, drugs, paper, production, and other industries, primarily confined to the extraction of phycocolloids **(Lahaye, 2001)** and some fine biochemicals. Macroalgae have also been used in practical aquaculture as natural feed supplements **(Mansilla & Ávila, 2011)**. Benthic macroalgae are vital components of marine ecosystems in relatively shallow coastal waters. Seaweed has recently gained prominence due to its bioactive components and uses **(El-Shenody *et al.*, 2019; Bowyer *et al.*, 2020; Hosseini *et al.*, 2020; Davies *et al.*, 2021)**.

Padina species is a rich source of fatty acids, ashes, carbohydrates, dietary fibers, proteins and lipids **(Subramanian *et al.*, 2015)**. *Padina pavonica* has exceptional potential to produce biochemicals, antioxidants, and various metabolites. It has received attention from researchers for its utilization as an antibiotic, antioxidant, antimicrobial, hepatoprotective, hypo-allergenic, anti-inflammatory, antidiabetic, and as a good source of food, fodder, plant growth promoter and bio-fertilizer **(Ansari & Ghanem, 2019)**

Rabbitfish, *Siganus rivulatus*, is one of the tasty white meat, and it is a herbivore, responsive to artificial food, and easy to spawn so that demand for fry stocks can be met through hatcheries **(Lante & Syah, 2007; Syah *et al.*, 2007)**. Rabbitfish can survive in high stocking density and quickly adapt to salinity ranges between 5-40 g/L; hence, it has become a candidate promising species to be cultivated **(Nelson *et al.*, 1992; Darsono, 1993; Teitelbaum *et al.*, 2008; Gonzales *et al.*, 2018; Seale and Ellies, 2019; Syah *et al.*, 2020).**

Epidemics of bacterial diseases are common in aquaculture. Predisposition to such bacterial outbreaks is frequently associated with poor water quality, organic loading of the aquatic environment, handling, fish transport, marked temperature changes, hypoxia, or other stressful conditions. One of the common pathogenic bacteria affecting fish is *Pseudomonas* sp. (such as *P. anguilliseptica*); it is the causative agent of 'Selutenbyo,' or red spot disease, of pond-cultured Japanese eel *Anguilla japonica* **(Wakabayashi & Egusa, 1972)**. *P. anguilliseptica* causes petechial hemorrhages in skin, peritoneum, and liver **(Magi *et al.*, 2009)**. In contrast, **Wiklund and Bylund (1990)** showed that antibiotic therapy with oxytetracycline has not attained success.

The current study investigated the effect of marine macroalga *P. pavonica* as a natural dietary feed additive on growth, histological status, and resistance of rabbitfish *S. rivulatus* fry to *Pseudomonas anguilliseptica* bacteria.

2. MATERIALS AND METHODS

2.1. Collection and preparation of *P. pavonica* alga:

The macro-alga species were identified depending on their morphology using taxonomic references, and it was found that it was *P. pavonica* belonging to the

Phaeophyta family **(Sahoo, 2001).** The *P. pavonica* macro-alga species were collected from Marsa Allam, the Red Sea coast, Egypt. It was washed with seawater at the sampling site to remove sediments and impurities and put in polyethylene bags. At the laboratory, algal samples were rinsed with distilled water to remove the remaining impurities and epiphytes. After that, the algae were dried in an oven at 50^{o}C till a constant mass was obtained **(Pereira *et al.*, 2012)**. The dried algae were crushed, powdered, and sieved using an electrical shaker. The powder was packaged in polyethylene bags and maintained in a refrigerator (- 4 ^{o}C) till future use. 1.5 -2.0 size particles mm were used. According to **Asma *et al.* (2013)**, **Kerzabi-Kanoun *et al.* (2021**) and **Shams El-Din *et al.* (2022)**, the alga constituents were determined and calculated (Table 1.).

Table 1. Biochemical and bioactive content, moisture, ash, and fibers (DM %) contents in *P. pavonica*

Biochemical composition	***P. pavonica***
crude Proteins	5.90±0.62
Lipids	2.83±0.58
Ash	42.16±2.20
Crude Fibers	19.25±3.48
Total Carbohydrates	29.86±2.62
Moisture	26.89±0.20
Gross energy (GE, Kcal/100 g)	182.46
Chemical constituents of *P. pavonica* liquid extract (mg/L).	
Nitrogen [a]	10.90
Phosphorus [a]	9.26
Potassium [a]	160.13
Calcium [a]	110.22
Magnésium [a]	1.20
Sulfur [a]	235.00
Sodium [a]	73.40
Chloride [a]	85.09
Manganese [a]	0.22
Carbonate (HCO_3^-) [a]	207.40
Bioactive compound	%
Methanolic extract [b]	4.18
Ethyl acetate fraction [b]	1.86
Butanolic fraction [b]	0.48
Tannins [b]	0.40
Saponins [b]	0.25
Polysaccharides [b]	3.48
Total phenolic, flavonoid, and proanthocyanidin contents (Methanolic Extract)	
Total phenolics (mg GAE/g DM) [b]	2.007 ± 0.104
Total proanthocyanidins (mg CE/g DM)[b]	4.611 ± 0.346
Total flavonoids (mg CE/g DM) [b]	1.132 ± 0.091

Values represent the means of three replicates ± standard deviations on a dry weight basis

[a] **(Asma *et al.*, 2013)** [b] **(Kerzabi-Kanoun *et al.*, 2021)**

The total carbohydrate content was assessed according to the phenol-sulphuric acid method for total carbohydrates **(DuBois *et al.*, 1956)**. Total protein content was measured according to the process of **Lowry *et al.* (1951)**, using salt-free bovine serum albumin as a standard. Total lipids content was estimated according to chloroform-methanol (2:1 by volume) for extraction as described in the study of **Folch *et al.* (1957)**. The results were expressed as mg/g of dry weight.

The moisture content of the algae was determined by drying 2g of samples in a thermo-regulated incubator (Lab Companion IB- G Series air, CHINA) at 105°C until reaching a constant weight **(AOAC, 1995)**. Ash content was gravimetrically assessed after heating samples in a muffle furnace at 550°C overnight. Then, the result was expressed as a percentage of dry weight. Fibers were quantified on 2g samples previously boiled with dilute H_2SO_4 (0.3 N) according to **Marinho-Soriano *et al.* (2006)**.

2.2. Diets preparation

Four isonitrogenous (32% crude protein) and iso-lipid diets (9% total lipid) were used in the current study, following **Wang *et al.* (2010)** who observed that the optimum requirements of *Siganus canaliculatus* for protein and lipid were about 32% and 8–9%, respectively. The control diet was enriched with *P. pavonica* (PP) at levels of 0.0 (control, T_0), 50 (T1), 75 (T2), and 100 (T_3) g/kg diet (Table 2). Then, the PP was suspended in 100-mL distilled water, added to diet ingredients by uniform spraying, mixed well for 30 min and pelleted (1–2 mm diameter) **(Abdel-Tawwab *et al.*, 2022)**. The prepared diets were stored in plastic bags at −4°C for further use.

2.3. Experimental fish and rearing conditions

The investigation was conducted at the Gulfs of Suez & Aqaba's Branch, National Institute of Oceanography and Fisheries (NIOF), Suez, Egypt. Fry rabbitfish specimens, *S. rivulatus*, were collected from the Gulfs of Suez, Suez, Egypt, and fish individuals were acclimatized to laboratory conditions in a rectangular tank for two weeks. After the acclimatization period, 600 healthy rabbitfish fry (1.10±0.06 g) were distributed into 12 cylindrical fiberglass tanks (500 L) at a rate of 50 fish/tank, representing four treatments in triplicates. The tanks were supplied with seawater sources. Aeration was continuously provided using an air blower. The daily water exchange rate was 30 % of the total tank volume.

Table 2. Feed formulation and proximate chemical composition analysis (% on dry matter basis) of the experimental diets containing different levels of macroalgae *P. pavonica*

	Experimental diets (g/kg)			
Ingredient %	**Control (T_0)**	**T_1**	**T_2**	**T_3**
Fish meal (CP 60%)	270	270	270	270
Soybean meal (CP 44%)	290	290	290	290
Corn meal (CP 7%)	210	160	135	110
Rice bran (CP 12%)	150	150	150	150
Padina pavonica (CP 5.90%)	0	50	75	100
Fish oil	40	40	40	40
Vitamin mix[1]	20	20	20	20
Mineral[2]	20	20	20	20
Total	1000	1000	1000	1000
Chemical composition %				
Dray matte %	93.26	92.31	92.65	92.54
Crude protein %	32.42	32.34	32.12	32.1
Ether extract %	9.2	9.34	9.44	9.51
Crude fiber %	4.84	5.32	5.65	6.17
Ash %	6.77	6.23	6.41	6.65
Nitrogen-free extract (NFE) % [3]	46.77	46.77	46.38	45.57
Gross energy (MJ/100 g diet) [4]	1.932	1.936	1.928	1.917

[1] Each one Kg of vitamin mixture contained: Vit. A 72000IU, Vit. B_1 6 mg, Vit. B_3 12000 IU, Vit. B6 9 mg, B12 0.06 mg, Vit E 60 mg, Vit. 12 mg, Pantothonic acid 60 mg, Nicotinic acid 120 mg, Folic acid 6 mg, Biotin 0.3 mg and Choline chlorids 3mg.

[2] Each one Kg of the mineral mixture contained: Zinc sulfate heptahydrate 3.0, Mg, sulfate 0.335, Coppous chloride 0.10, Calcium phosphate monobasic 135.8, Calcium Lactate 327.0, Ferric citrate 29.7, Potassium phosphate dibasic anhydrous 239.8, Sodium phosphate monobasic 87.2, Sodium chloride 43.6, Aluminium chloride anhydrous 0.15, Potassium iodide 0.15, Cobalt chloride 1.0, Sodium selenite 0.011 and L-cellulose 132.25 (as g/Kg mineral mix) **Gatlin and Wilson (1984).**

[3] Nitrogen-Free Extract (calculated by difference) = 100 – (protein% + lipid% + ash% + fiber%).

[4] Calculated according to **NRC (1993)** using factors 5.65 k.cal/g for protein, 9.45 k.cal/g for fat, and 4.1 k.cal/g for carbohydrate. 1 kcal, = 0.004184 MJ

During the experimental trial, water temperature was recorded at a daily average of 28±2 °C. The dissolved oxygen (DO) was monitored weekly using a portable DO meter (YSI, model Pro20, USA), with an average of 5±1 mg/L. Total ammonia-N was

measured once a week and was less than 0.16 ± 0.01 mg/L, salinity averaged 36.00 ± 2.00 g/L as determined by a refractometer, and pH average was 7.50±0.3 being measured by a digital multi-meter (Crison, model MM41, Spain).

After the feeding period (eight weeks), fish were starved for one day before sampling and anesthetized with 100 mg Tricaine Methanesulfonate (MS222)/L. All fish in each tank were independently counted and weighed to estimate growth indices as follows:

Weight gain (g) (WG) = final weight (FW) – initial weight (IW);

Specific growth rate (SGR; % /day) = 100 [ln FW (g) – ln IW (g)] / trial period (days);

Feed conversion ratio (FCR) = total dry feed intake (g) / weight gain (g);

Fish survival (%) = 100 × (fish number at the end/fish number at the beginning).

2.4. Tissues sampling

At the end of the experiment, three fish were randomly sampled from each tank, killed by the medullary section and dissected. The intestine, liver, and splain were removed and immediately stored (10% formalin) for histopathological evaluation. The procedures were performed following the protocol approved by the Ethics Committee on the Use of Animals of Londrina State University, according to Brazil's National Council for the Control of Animal Experimentation.

2.5. Proximate chemical composition of fish body

The proximate composition of fish samples (initial and final, with five fish per tank) was determined according to **AOAC (1995)**. Crude protein was determined using the micro-Kjeldahl method, N% × 6.25 (using a Kjeltec Autoanalyser, Model 1030, Tecator), and crude lipid using Soxhlet extraction with diethyl ether (40–60°C; Soxtec System HT6, Tecator. Dry matter was resolved after oven drying (105 °C) for 24 h (Drying Oven, GE-174, Memmert). At the same time, ash was measured by incineration at 550 °C for six h (Thermo Scientific Heraeus M110 Muffle Furnace).

2.6. Challenge test with pathogenic bacteria

A virulent strain of *P. anguilliseptica* bacteria was initially isolated from diseased fish in the central laboratory, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Bacterial isolates were frozen at –70 °C in Tryptic Soy Broth (TSB-1), supplemented with 1% NaCl and 15% (v/v) glycerol until used **(Romalde 1999)**. At the end of the feeding trials, 20 fish from each group were transported to 100-L aquaria in duplicates (10 fish/aquarium) and injected intraperitoneal (IP) with 0.2 mL of the pathogenic bacterial suspension with a dose of 2×106 CFU/mL. The other twenty fish (10 fish/aquarium) were injected with pure saline at the same dose of the other groups and used as a negative control. Fish in each treatment fed on the corresponding diets, as previously mentioned, were observed for 14 days, during which any clinical signs and mortalities were daily recorded to calculate the mortality rate.

2.7. Histological Status

The intestine, liver, and splain samples were fixed with 10% of formalin and embedded in paraffin. Sections of 3µm thickness were submitted from each block,

mounted to a glass slide, stained by hematoxylin and eosin (H&E), and examined by an independent pathologist. Slides were scanned, and images were processed using an image scanner and viewer software (LOCI, University of Wisconsin, US).

Histopathological changes for intestinal tissue assessed the degree of inflammation and scored it from 0 to 4 (0, normal; 1, mucosal hyperplasia; 2, spotty infiltration by inflammatory cells not involving the entire mucosa and/or submucosal thickness; 3, marked increase of inflammatory cells involving the full thickness of mucosa and/or submucosal thickness; 4, marked increase of inflammatory cells in BOTH mucosa and submucosa.

2.8. Statistical analysis

One-way ANOVA was used to evaluate dietary PP supplementation's effects on different parameters. Duncan's multiple range test was used as a post-hoc test to assess the differences between means, and the value (P<0.05) was considered statistically significant **(Duncan, 1955)**. The optimal dietary supplementation level of marine macroalga was estimated using polynomial regression analysis following **Yossa and Verdegem (2015)**. Statistical analysis was done by SPSS program version 26 **(Dytham, 2011)**.

3. RESULTS

3.1. Growth performance

Dietary supplementation with the PP macroalga recorded a positive effect on all growth metrics in a supplementation level-dependent manner, as they increase significantly (P<0.05) with an increase in *P. pavonica* levels (Table 3). The highest substantial (P<0.05) values of FW, WG, FI, FCR, and SGR were recorded in the T_3 group, compared to the control group that showed the lowest values. Growth, feed utilization, and survival (%) significantly improved with increasing PP rate in all experimental fish diets, compared to the control group (Table 3).

Table 3. Growth performance and feed utilization of rabbitfish fed diets supplemented with various levels of marine macroalga *P. pavonica* for 8 weeks

P. pavonica g/kg diet	IBW	FW (g)	WG% (g)	FI (g)	FCR	SGR (% day $^{-1}$)	Survival %
0 (T_0)	1.10±0.06	6.38±0.01 c	5.38±0.01 c	10.50±0.34 c	1.95±0.06 a	3.14±0.00 c	96.3±0.67
50 (T_1)	1.07±0.07	8.00±0.35 b	7.00±0.35 b	12.13±0.21 b	1.74±0.06 b	3.59±0.03 b	95.3±0.67
75 (T_2)	1.10±0.06	8.29±0.03 b	7.29±0.03 b	12.68±0.24 b	1.74±0.03 b	3.61±0.00 b	96.7±0.66
100 (T_3)	1.10±0.06	11.67±0.04 a	10.67±0.04 a	13.89±0.42 a	1.30±0.04 c	4.22±0.00 a	98.0±1.15

The means having different letters in the same column are significantly different at P < 0.05.

Fish-provided diets contained macroalga ingested more feed than those fed on the control diet. The highest FI was observed in fish groups fed the 10 g PP/kg diet (13.89 g /fish), while fish fed on the control diet ingested the least feed (10.50 g /fish). With

increasing dietary PP levels, a significant improvement in FCR values was observed, with the best values recorded in the T3 treatment (1.3), compared to the control group (1.95). Interestingly, fish in all experimental groups were in good health during the feeding time, and their survival (%) ranged from 95.3% to 98% (Table 3). The second-order polynomial regression between FW, SGR, WG %, FI, and dietary PP levels are shown in Fig. (1).

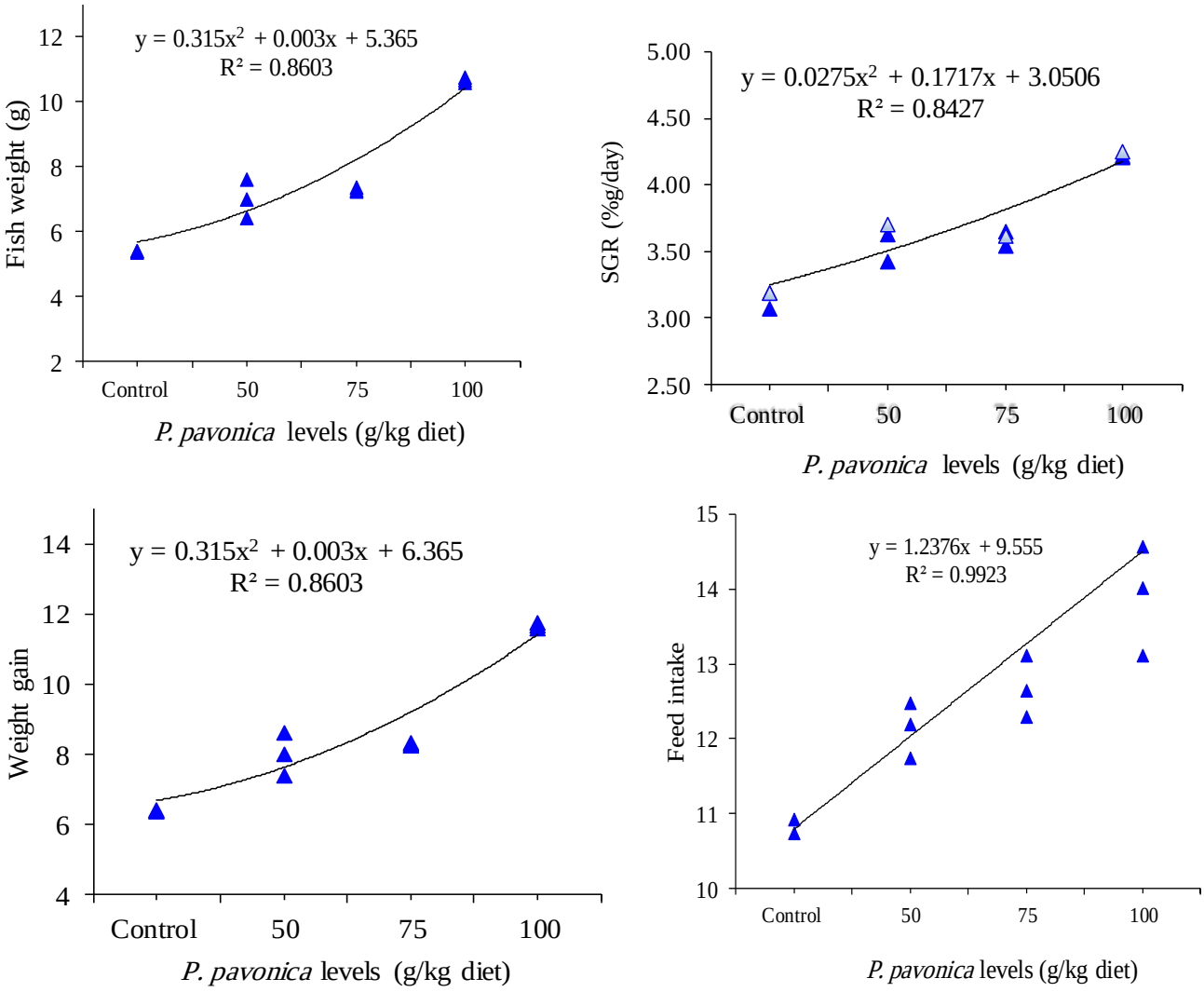

Fig. 1. The polynomial regression analysis between final fish weight (g), specific growth rate (SGR; %/day), weight gain(g), and feed intake (g feed/fish) of rabbitfish and different dietary levels of marine macroalga *P. pavonica*

3.2. Fish body composition

Proximate chemical analysis of rabbitfish indicated that moisture, crude protein, and total lipid were significantly ($P < 0.05$) impacted by dietary PP, while ash content was not considerably affected ($P > 0.05$; Table 4). With increasing dietary PP levels, total body protein content increased. The opposite trend was observed for total body lipid and moisture content. The highest significant ($P < 0.05$) values of moisture, total lipid, and ash contents were observed in the control group (75%, 6.57%, and 3.6 %, respectively), while the lowest ones were observed in fish fed 100g PP /kg diet (71.33%, 4.83%, and 3.13 %, respectively). On the other hand, the highest and lowest values of crude protein content were recorded for fry fed T_3 diet and the control group (16.10% and 13.37 %, respectively).

Table 4. Proximate chemical composition of carcass rabbitfish fed diets (% as wet weight basis) supplemented with various levels of marine macroalga *P. pavonica* for 8 weeks

P. pavonica (g/kg diet)	Moisture	Crude protein	Total lipids	Ash
0 (T_0)	75.00±0.58 a	13.37±0.09 d	6.57±0.50 a	3.60±0.12
50 (T_1)	74.00±0.58 a	14.40±0.15 c	5.50±0.35 b	3.43±0.28
75 (T_2)	73.33±0.33 ab	14.90±0.06 b	5.20±0.12 b	3.27±0.07
100 (T_3)	71.33±0.88 b	16.10±0.17 a	4.83±0.12 b	3.13±0.15

The means having different letters in the same column are significantly different at $P<0.05$.

3.3. Histopathological investigations

No abnormal or histological changes were detected in sections of the intestine, liver, and spleen of rabbitfish fed on diets supplemented with different levels of *P. pavonica*. Intestine histology of all groups showed uniform intestinal tissue with regular villi (Black arrows, Fig. 2). Control group T0 showed uniform intestinal tissue with regular villi (Black arrows). Intestinal tissue of the T1 group (50 g PP/kg diet) showed a significant reduction in the height of the villi (Black arrows) with chronic inflammatory cells, while intestinal tissue of the T2 group (75 PP g/kg diet) showed regular villi with few congested vessels within lamina properia. Intestinal tissue of group fed 100 PP g/kg diet displayed uniform intestinal tissue with normal villi.

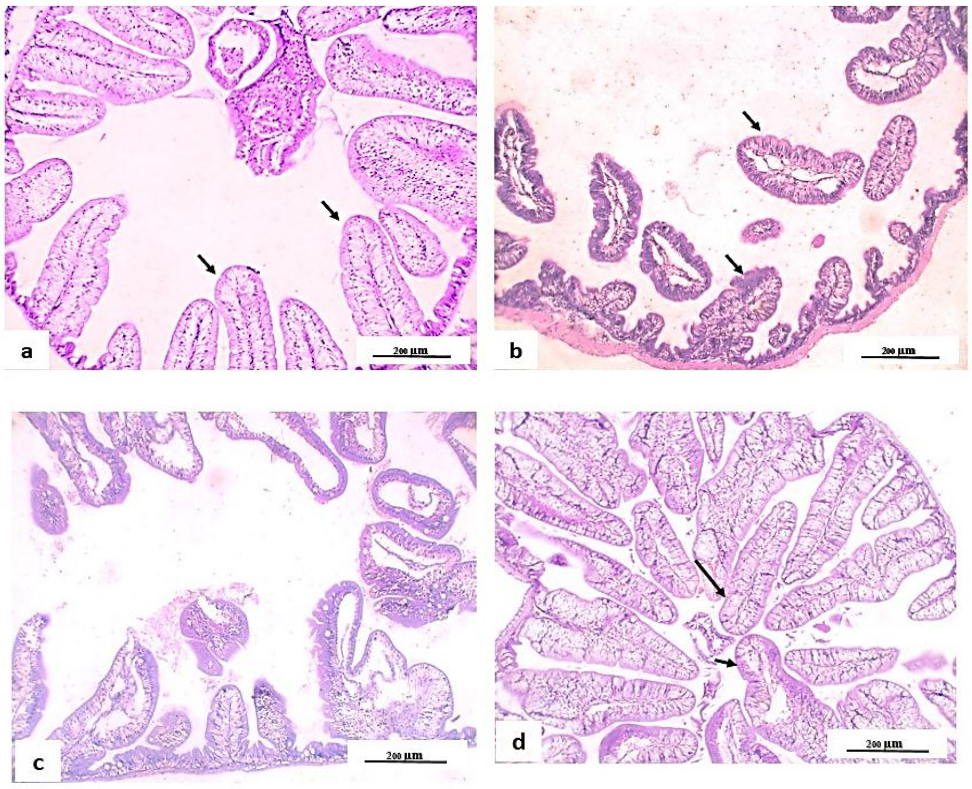

Fig. 2. The effect of different dietary levels of marine macroalga *P. pavonica* on the intestine of rabbitfish after 8 weeks of feeding

Letters a,b,c, and d represent the intestine for T0, T1, T2, and T3 (10 X).

Liver histology exhibited consistent liver tissues in all treatments, with uniform hepatocytes with no evidence of injury (Arrowheads) and uniform portal tracts (Fig 3). The control group showed uniform liver tissue, hepatocytes, and portal tracts with no evidence of injury (Arrowheads), while the liver tissue of the group fed 50g/kg, showing moderately congested vessels with inflammatory cells (Black arrows). Fish fed 75g PP/kg diet showed uniform liver tissue and hepatocytes, with no evidence of injury (Arrowheads), and liver tissue of group fed 100g/kg showed uniform liver cells with normal hepatocytes.

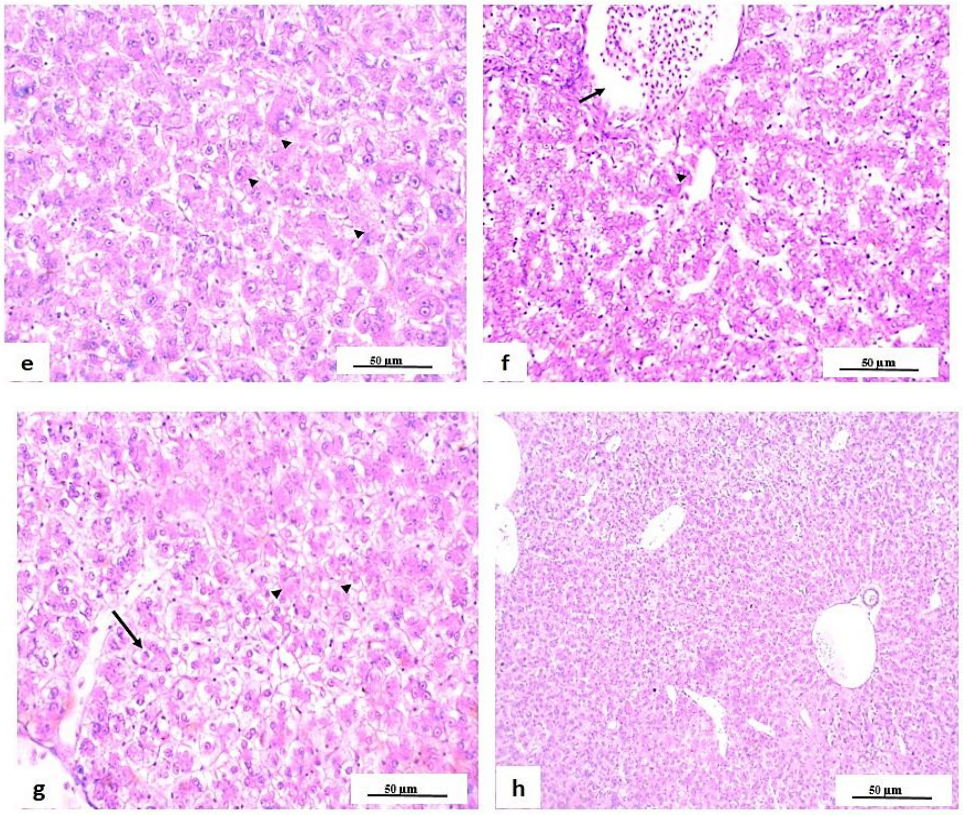

Fig. 3. The effect of different dietary levels of marine macroalga *P. pavonica* on the liver tissue of rabbitfish after 8 weeks of feeding

Letters e, f, g, and h represent the liver for T0, T1, T2, and T3 (40 X).

The spleen tissues also showed consistent splenic tissue, white pulp (Black arrows), and red pulp (Red arrows) at all treatments. No evidence of hemorrhage or hemosiderin-laden macrophages had been found (Fig 4). The control group showed uniform splenic tissue, white pulp (Black arrows), and red pulp (Red arrows). Spleen tissue of group fed 50g/kg, showing the marked expansion of red pulp (Red arrows) and hemosiderin-laden macrophages. (k) Fish fed 75g/kg (T2), showing uniform splenic tissue, white pulp (Black arrows), and red pulp (Red arrows), while the T3 group, showing the marked expansion of red pulp (Red arrows), still remnant of white pulp (Black arrow). No evidence of hemorrhage or hemosiderin-laden macrophages.

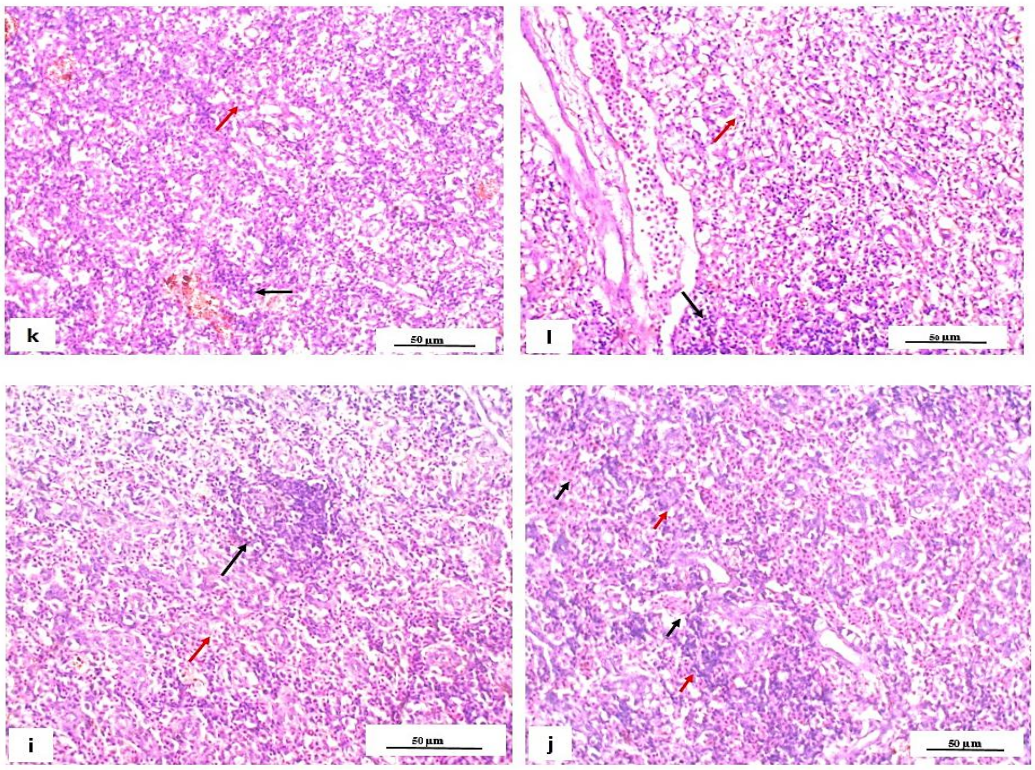

Fig. 4. The effect of different dietary levels of marine macroalga *P. pavonica* on the spleen of rabbitfish after 8 weeks of feeding

Letters i,j,k, and l represent the spleen for T0, T1, T2, and T3 (40 X).

3.4 Challenged fish mortality (%)

The present results revealed that fish fed on PP-enriched diets significantly (P<0.05) improved fish health against *P. anguilliseptica* infection. Rabbitfish death after 14 days of infection was considerably decreased in a dose-dependent way, and its cumulative mortality was at its highest in the control group (72.5%), while the lowest one (22.5%) was observed in the group fed 100 g PP /kg feed (Fig. 5).

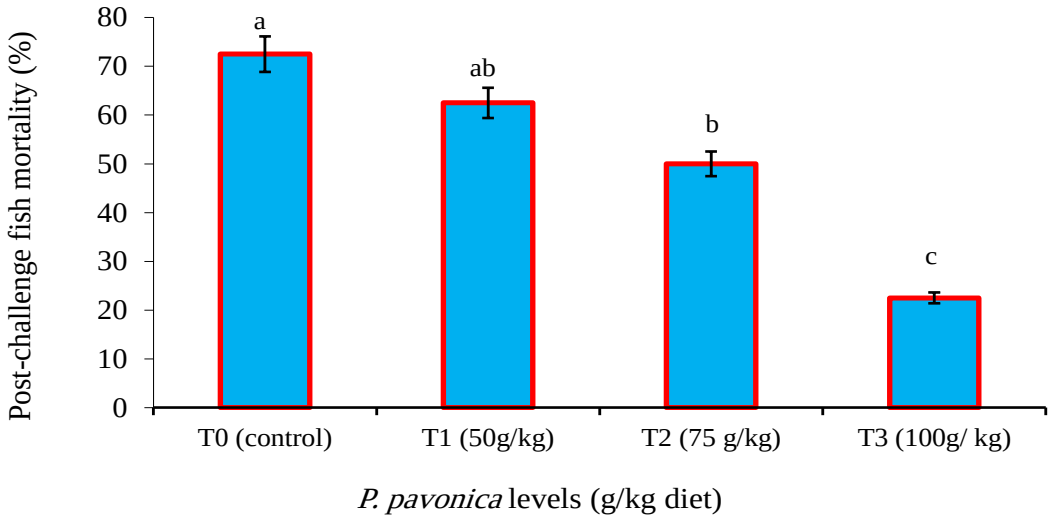

Fig. 5. Mortality (%) in rabbitfish fed different levels of marine macroalga *P. pavonica* for 8 weeks and post-challenged by *P. anguilliseptica* bacterial infection for 14 days
Bars having different letters are significantly different at $P < 0.05$.

4. DISCUSSION

4.1. Growth performance

Algae including *P. pavonica* are rich in pigments, proteins, lipids, fatty acids, minerals, nutrients, vitamins, ascorbic acid, phytohormones, antioxidants, and other bioactive substances **(Hamed *et al.*, 2018; Ashour, 2019; Peixoto *et al.*, 2019; Safavi *et al.*, 2019; Sajina *et al.*, 2019; Sharawy *et al.*, 2020)**. These bioactive ingredients enhanced growth performance, feed utilization, carcass composition, blood parameters, immunity response, and antioxidant and antibacterial properties **(Ashour *et al.*, 2018; Sharawy *et al.*, 2020)**. In the current study, macroalga *P. pavonica* supplementation to rabbitfish significantly improved all growth parameters and feed utilization. The current finding may be due to the alga having high contents of bioactive components such as polyphenols and various polysaccharides, viz. alginates, laminarins and fucoidans **(Lee, 2008)**. The biochemical analysis of *P. pavonica* showed that it contains 3.48 % polysaccharides **(Kerzabi-Kanoun *et al.*, 2021)**. Polysaccharides have demonstrated positive responses on growth performance, feed utilization, serum biochemical composition, immune responses, and disease resistance for many cultured aquaculture species **(Van Doan *et al.*, 2017; Akbary & Aminikhoei, 2018; Yengkhom *et al.*, 2018)**.

Carbohydrates, proteins, and lipids are essential in different biochemical processes **(Wang *et al.*, 2004)**. The current study's proximate analyses of *P. pavonica* showed that it contains 29.85% carbohydrates, 5.90% protein, and 2.83% lipid, in addition to 19.5% fiber (Table 1). Our finding is close to that recorded in previous studies, with 1-5% or 1.82-3.01% for lipids **(El-Shoubaky, 2008; El Maghraby &**

Fakhry, 2015), 5-7% for protein **(Subramanian *et al.*, 2015)** and 25-39 % for carbohydrates **(Ansari *et al.*, 2019)**. Moreover, *P. pavonica* collected from the study area recorded a high percentage of ashes, water content and fibers (42.16, 26.89, and 19.25 %), respectively; this result agrees with that of **Ansari *et al*. (2019)**. Fibers play a severe role in the movement of the intestines **(Dreher, 2018)**. These observations are essential as microalgae are used as feed for aquatic animals, and these characterizations help assess the suitability required for enhancing the nutritional value of diet and improving the FCR **(Suresh Kumar *et al.*, 2015; Ashour *et al.*, 2018)**. Using algae and their derivatives in feeding aquatic animals resulted in improved growth performances alongside with an innate immunity enhancement **(Sharawy *et al.*, 2020)**. Furthermore, many studies have postulated that diets supplemented with seaweeds/algae positively enhance growth performance, feed utilization, FCR, and survival rate in many fish species **(Güroy *et al.*, 2007; Ergün *et al.*, 2009; Wassef *et al.*, 2013; Khalafalla & El-Hais, 2015)**.

4.2. **Fish body composition**

Data of this experiment exhibited that fish fed with PP-enriched diets recorded relatively higher dry matter and protein contents along with lower lipids content than those of the control diet (Table 4). Carcass protein content in the present study increased, while lipid content decreased gradually with rising PP levels in the diet to 100 g/kg diet, which agrees with the results of **Azaza *et al.* (2008)** who verified that, increasing supplemental levels of seaweed meal decreases carcass lipid content of the Nile tilapia. Seaweed has a good vitamin and mineral content and is especially rich in ascorbic acid or vitamin C **(Ortiz *et al.*, 2006; Valente *et al.*, 2006)**. Vitamin C is a promoter of lipid metabolism, which may affect body metabolism and composition **(Kotze, 1975)**. The reasons mentioned earlier may alter body nutrient deposition in fish, decrease carcass lipid, and save a protein nutrient for tissue development **(Miyasaki *et al.*, 1995; JI *et al.*, 2003)**. Additionally, many studies have shown that diets supplemented with seaweed enhanced carcass composition in fish **(Güroy *et al.*, 2007; Ergün *et al.*, 2009; Wassef *et al.*, 2013; Khalafalla & El-Hais, 2015)**.

4.3. Fish resistance to *P. anguilliseptica* infection

The results of the present study indicate that *P. pavonica* had a protective consequence against *P. anguilliseptica* infection in fish (Fig. 5). The brown alga *P. pavonica* is well-recognized for its medical value **(Rajasulochana *et al.*, 2009; Ansari *et al.*, 2019)** since they may contain physiologically active metabolites that are not present in other species and are employed in the pharmaceutical industries. Brown macroalga *P. pavonica* generates antimicrobial components essential in suppressing the growth of many pathogens in humans and other animals **(Morais *et al.*, 2020)**. These antioxidant molecules are created in their body tissues in response to harsh environmental circumstances **(Matanjun *et al.*, 2008)**. *P. pavonica* has hepatoprotective, hypolipidemic, antioxidant, and anti-inflammatory properties and can be utilized to treat hepatotoxicity

(Ahmed *et al.*, 2016). Antioxidants are essential in protecting shrimp and fish tissues from oxidative damage and are usually affected by feed composition **(Sharawy *et al.*, 2020)**. In addition, polysaccharides in *P. pavonica* play a vital role in immune-stimulatory and disease resistance **(Akbary & Aminikhoei, 2018).**

P. pavonica can develop new antibacterial substances alone or in collaboration with antagonistic bacteria **(Ismail et al., 2016; Ansari *et al.*, 2019)**. Brown algae extracts have anticoagulants **(Chevolot *et al.*, 2001)**, anti-thrombogenic, antitumor **(Maruyama *et al.*, 2006)**, anti-inflammatory **(Cumashi *et al.*, 2007)** antiviral, and antioxidant properties in addition to immunomodulatory properties **(Ahmed *et al.*, 2016)**. Many studies have shown that a diet supplemented with seaweed enhances disease resistance in fish **(Güroy *et al.*, 2007; Ergün *et al.*, 2009; Wassef *et al.*, 2013; Khalafalla & El-Hais, 2015).**

4.4. **Histopathological status**

The current experiment showed no histopathological changes among the control and PP-treated groups (Fig 2, 3, 4). This finding coincides with that of **Hussein *et al.* (2013, 2017)**, who deteced no histopathological changes between the control and treated groups in the liver and intestine samples of *O. niloticus* fed on seaweed *Taonia atomaria* supplemented diets. Similarly, no abnormal or histological changes were detected in sections of the liver, stomach, and intestine tissues of the red tilapia fed on different levels of dietary polysaccharides derived from brown macroalga *Sargassum dentifolium* **(Abdelrhman *et al.*, 2022)**. The present findings concur with results recorded in previous studies **(Lyons *et al.*, 2017; Sotoudeh and Mardani, 2018; Krogdahl *et al.*, 2021)**, which found that rainbow trout-fed diets supplemented with either microalga or red seaweed showed no abnormal in the liver, stomach or intestine tissues.

5. CONCLUSION

The macroalgal *P. pavonica* is a rich source of polysaccharides, carbohydrates, and proteins; therefore, it plays a vital role in fish growth and health. The current study concluded that using *P. pavonica* as a feed supplement for rabbitfish, *S. rivulatus* fry significantly enhanced growth performance, feed utilization, FCR, and immune responses against *P. anguilliseptica* bacteria.

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