Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(6): 225 – 242 (2023)
www.ejabf.journals.ekb.eg

Dietary Effect of Microalga *Padina pavonica* Richness with Fatty Acids on the Growth and Immune Status of *Coptodon zilli*

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ARTICLE INFO

Article History:
Received: Oct. 3, 2023
Accepted: Oct. 25, 2023
Online: Nov. 29, 2023

Keywords:
Brown alga,
Fatty acids,
GC-Mass,
Immune response,
Redbelly tilapia

ABSTRACT

The fast growing of aquaculture sector increases the needs of natural feed substances such as microalgae. The present study was performed to determine fatty acids composition of brown alga *Padina pavonica* and address the effects of dietary *P. pavonica* on growth performance, body composition, bio-somatic indices, blood parameters, antioxidant activity, and oxidative stress marker of redbelly tilapia *Coptodon zilli*. Redbelly tilapia specimens (3.58 ± 0.02g) were fed with commercial pellets mixed with four concentrations of *P. pavonica* 2.0, 4.0, 6.0, and 8.0g/ kg for 45 days. This study was performed under farming conditions. The results showed that the oleic acid C18:1(ω9) is the most dominant constituent of fatty acids in *P. pavonica* as monounsaturated fatty acids. Compared to the other groups, Σ MUFAs showed the maximum percentage. At the end of the trial, a highly significant difference was detected between groups in terms of growth performance, body composition, blood indices and somatic indices. In addition, between groups, a highly significant difference ($P \leq 0.05$) was recorded concerning MDA, SOD and GSH, while no significant difference ($P > 0.05$) was shown by CAT enzyme. Generally, the highest results were recorded with concentrations (6.0 and 8.0g/ kg) of *P. pavonica* diets, as compared to their control.

INTRODUCTION

Coptodon zillii, previously known as *Tilapia zilli*, is one of the most widely distributed species found. It has been recorded in the Red Sea **(Bayoumi, 1969)**, freshwater, brackish lakes, the Mediterranean Sea **(Moharram & Akel, 2007)** and River Nile **(El-Bokhty & El-Far, 2014)**. The broad useful effects of using algae in fish nutrition are not only reflected in a positive impact on growth and health status but also significantly enhance specific biological and physiological activities **(Nahavandi *et al.*, 2023)**. In the last few decades, aquaculture has massively proliferated food production depending on good aquafeeds introduced for fish farming, which are considered the main cost in fish farming

(Bahi *et al.*, 2023). Microalgae have many benefits (rich in important bioactive substance-protein, amino acids, polysaccharides, unsaturated fatty acids), which make them a valuable source of feed additive **(Li *et al.*, 2015)**. Algae and other plant-based feed additives can increase feed consumption **(Gabriel *et al.*, 2015)**, improve immune system **(Bilen *et al.*, 2016)**, enhance fish growth **(Doan *et al.*, 2020)**, improve digestion by boosting the secretion of various digestive enzymes **(Xu *et al.*, 2020)**, stengthen disease resistance **(Abdel-Tawwab & El-Araby, 2021)**, and reduce stress **(Yousefi *et al.*, 2021)**.

The following study was conducted to evaluate fatty acids composition of the brown alga *Padina pavonica* and determine the effect of different concentrations of *P. pavonica* (2.0, 4.0, 6.0 and 8.0 g./kg), added to commercial feed, on the growth performance and immune status of cultured *C. zilli* juveniles.

MATERIALS AND METHODS

1. Ethical statement

All the experimental protocols including lab animals were carried out according to NIOF committee for Ethical Care and Use of Animals and Aquatic Animals (NIOF/ IACUC), with an approval number (NIOF/AQ2/F22/R028).

2. Collection of brown algae *(Padina pavonica)* and preparation

Fresh brown alga *P. pavonica* samples were collected from Marsa Allam costal water, the Red Sea, Egypt, during summer 2020. Based on its morphology, the identification of the macroalgae species was provided according to **Sahoo *et al.* (2002)**, then processed to powder according to **Mohsen *et al.* (2007)**. Chemical composition of *P.pavonica* was assessed according to **Maghawri *et al.* (2023)**.

3. Determination of fatty acids composition

3.a. Preparation of methyl ester of fatty acids

The transmethylation of lipids and extraction of fatty acid methyl esters (FAMEs) were prepared from aliquots comprising total lipids, as mentioned by **Radwan (1978)**, and then they were stored at 4°C in the dark before GC–MS analysis.

3.b. Gas chromatography–mass spectrometry (GC-MS) analysis

GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) was used for the analysis of the fatty acid methyl esters (FAMEs). Acquisition parameters were a direct capillary column TG–5MS (30m x 0.25mm x 0.25µm film thickness). The column oven temperature was initially set at 50°C and then increased by 5°C/ min to 250°C and held

for 2min, then it was increased to the final temperature of 300°C by 30°C/ min and held for 2min. The injector and MS transfer line temperatures were kept at 270 & 260°C, respectively. Helium was used as a carrier gas at a constant flow rate of 1ml/ min. The solvent delay was 4min, and diluted samples of 1µl were automatically injected using autosampler AS1300, coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C. The components were identified via comparing their mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

4. Diet preparation

Diets for treated groups were prepared by mixing *P. pavonica* concentrations 0, 2.0, 4.0, 6.0, and 8.0 g/kg with commercial pellets (1.2 mm) and protein 38% (Skretting Egypt Company), following the methods of **Nazarudin *et al.* (2020)**.

Table 1. Feed and chemical composition of diet ingredients with *P. pavonica* doses

Ingredient	**Feed composition (%)**				
Fish meal	14.25	14.25	14.19	14.18	14.15
Soybean	51.05	50	49.01	47.97	46.95
Maize	17.70	16.75	15.80	14.85	13.9
Wheat	11	11	11	11	11
Fish oil	4	4	4	4	4
*Vitamin (mineral premix)	2	2	2	2	2
Padina pavonica (g/kg diet)	0.0	2.0	4.0	6.0	8.0
Ingredient	**Chemical composition (%)**				
Crude protein	38	37.24	36.48	35.72	34.96
Crude fat	6.24	6.11	5.99	5.86	5.74
Fiber	6.18	6.14	6.02	5.89	5.68
Ash	7.5	7.35	7.2	7.05	6.9
Nitrogen-free extract	41.94	43.16	45.3	45.48	46.72
Padina pavonica (g/kg diet)	0.0	2.0	4.0	6.0	8.0

*Vitamin (mineral premix) included the following (g/kg^{-1} mixture): ascorbic acid 120; retinyl acetate 0.67; cholecalciferol 0.1; thiamin 5.6; menadione 22; pyridoxine 4.5; riboflavin 12; calcium-pantothenate 14.1; p-aminobenzoic acid 40; biotin 0.1; folic acid 1.5; choline chloride 350; inositol 50; canthaxanthin 10; butylated hydroxytoluene 1.5; $CaHPO_4$, $2H_2O$ 29.5; Ca $(H_2PO_4)_2$, H_2O 217; $NaHCO_3$ 94.5; Na_2SeO 35; H_2O 0.011; KCi 100; Ki 0.2; $MgCl_2$ 63.7; $MgSO_4$ 34.3; $MnSO_4$ 2; $FeSO_4$ H_2O 10; NaCl 172.4; $CuSO_4$ 5; $ZnSO_4$ 10, and H_2O 0.4.

5. Experimental conditions and feeding trials

Total numbers of 150 apparently healthy cultured redbelly tilapia (*Coptodon zilli),* with average body weight of 3.58 ± 0.02g were collected from Fish Farming and Technology Institute (FFTI), Suez Canal University, Egypt, and then transferred alive to the research unit. Fish were divided into five equal groups in fiberglass tanks (10 fish/ tank). Each

group had three replicates cultured in a 50-liter glass tank. The tanks were filled with fresh water aerated continuously.

Fresh water parameters were in the range of 5.61– 7.10mg/ L dissolved oxygen, 21 to 24˚C temperature and 7.6– 8.2 pH along the experiment, while the concentrations of total ammonia nitrogen (TAN), were 0.12–0.15 (mg/L).

Fish were acclimated two weeks in fiberglass tanks and fed with commercial pellets till the start of the trial which was designed as follows: Control diet with no additives (C); group 1 (G1) with 2.0g/ kg; group 2 (G2) with 4.0g/ kg; group 3 (G3) with 6.0g/ kg, and group 3 (G4) with 8.0g/ kg. For 45 days, fish were fed previously prepared diets two times daily (9 am., and 3pm.) by hand at a rate of 3% of their body weight, seven days/ week. All diets were kept in a dark box in the refrigerator at 4˚C.

6. Growth performance parameters and fish body composition

All fish were weighed and recorded the final weight (FW), the body weight gain in grams (WG), specific growth rate (SGR %), average daily gain (ADG) and condition factor (K) were calculated according to **Awad and Awaad (2017)** as:

WG =FW–IW; SGR(%/day) = 100 (ln FW–ln IW)/ T; condition factor (K) = W/L^3 × 100, and ADG = FW–IW/T

Where, W= fish weight; L= fish length; IW=initial weight (g); FW=final weight (g); T= period (days), and ln **=** the natural log**.**

The whole body composition of *C. zilli* (protein, lipids, moister and ash) was determined for three fish from each group by using the standard methods mentioned by the Association of Official Analytical Chemists **(AOAC, 1997)**.

7. Hepatosomatic, spleenosomatic and gonadosomatic indices

After dissecting the fish, the internal organs as liver, spleen and gonads were extracted. Organs were weighed utilizing a digital sensitive balance. Hepatosomatic index (HSI), spleenosomatic index (SSI) and gonadosomatic index (GSI) were calculated by using the following equations according to **Pandit and Gupta (2019)**:

HSI= Liver weight (g.)/ fish weight (g.) ×100

SSI= Spleen weight (g.)/ fish weight (g.) ×100

GSI = Gonads weight (g.)/ fish weight (g.) ×100

8. Blood sampling

After a day of fasting, three fish individuals from each group were lightly anesthetized by using clove oil solution (50µl). Blood samples were collected from the caudal vein and separated into two tubes according to method of **Noga (2010)**.

9. Hematological parameters

Total red blood cells (RBCs), hemoglobin content (Hb), hematocrit test (Hct), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), total white blood cells (WBCs), lymphocytes, monocytes, granulocytes and platelets were recorded due to methods mentioned by **Tran-Duy *et al.* (2008)**.

10. Biochemical parameters

Serum total protein (TP) content was determined according to **Lowry *et al.* (1951)**. Calcium (Ca), globulin (GL), albumin (ALB), creatinine (CR), urea (U) and uric acid (UA) were all measured in serum as described by **Samadaii and Bahrekazemi (2020)**. Liver enzymes in serum (alanine amino transferase (ALT), aspartate amino transferase (AST)) were detected using commercial kits as described by **Reitmann (1957)**.

11. Evaluation of antioxidant enzymes activity and oxidative stress marker

Antioxidant enzymes activities were evaluated in liver tissue from all groups. The reagents were purchased from a bio-diagnostic company (Diagnostic and Research agency). Malondialdehyde (MDA, CAT no. 25. 29) was determined according to the classical method described by **Del Rio *et al.* (2003)**. Superoxide dismutase (SOD; SD 25.21) activity was assessed according to the method described by **Paoletti *et al.* (1986)**. Reduced glutathione (GSH) was evaluated using reduced glutathione assay kit (GSH, CAT no. GR 25.11). Catalase (CAT; CAT no. CA 25.17) activity was assessed according to the method described by **Beers and Sizer (1952)**.

12. Statistical analysis

Results were presented as means ± the standard error (SE), then assessed by one-way analysis of variance (ANOVA) using IBM SPSS statistics 20.0. Duncan's multiple-range test and descriptions were used to ascertain differences between groups, with significance at $P \leq 0.05$ according to **Dytham (2011)**.

RESULTS

1. Fatty acids analysis

Fatty acids composition of *Padina pavonica* are shown in Fig. (1) and table (2), which are differentiated between saturated, and (mono and poly) unsaturated fatty acids.

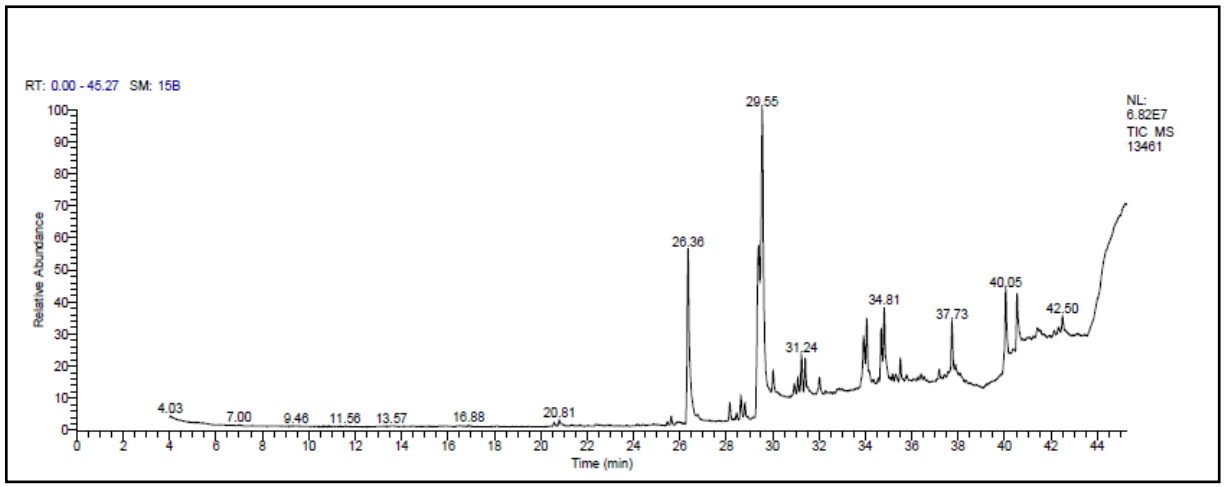

Fig. 1. Chromatogram of the FAMEs of *P. pavonica* by gas chromatography

Table 2. Fatty acids composition of *P. pavonica* (% of the total of fatty acid)

IUPAC	Common name	Number of carbon atoms	%
SFAs			
Palmitic acid	n-hexadecanoic acid	C16:0	14.58
n-Hexadecanoic acid ME	Palmitic Acid methyl ester	C17:0	0.59
	Octadecanoic acid	C18:0	1.67
MUFAs			
Oleic acid	9-Octadecenoic acid	C18:1(ω9)	18.94
Octadecenoic acid ME	9-Octadecenoic acid (Z)- ME	C19:1(ω9)	0.98
2-Oleoyl Glycerol	2-hydroxy-1-(hydroxymethyl)ethyl ester-9Z-octadecenoic acid	C21:1(ω9)	5.46
(13Z)-Docos-13-enoic acid	Erucic acid	C22:1(ω9)	3.49
Methyl (13E)-13-docosenoate 13-Docosenoic acid methyl	(Z)-Erucic Acid ME	C23:1(ω9)	3.49
PUFAs			
	Hexadecadienoic acid ME	C17:2(ω9)	2.76
Linolic acid	9E,12E-octadecadienoic acid	C18:2(ω9)	9.02
2- Linoleoyl glycerol	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester	C21:2(ω6)	5.34
Σ SFAs			19.6
Σ MUFAs			32.36
Σ PUFAs			17.12
Total			69.08

* Saturated fatty acids
**Monounsaturated
*** Polyunsaturated

2. Growth performance and somatic indices of *C. zilli* after feeding with *P. pavonica*

Growth performance parameters showed a highly significant difference between groups ($P \leq 0.05$). G3 group exhibited a significant increase compared to others, in terms of FW, TL, St. L, WG, ADG and SGR percentages. All somatic indices recorded a highly significant difference ($P \leq 0.05$). G3 group recorded highly significant means in HHI and SSI, while G3 registered a highly significant SSI means (Table 3).

Table 3. Effect of dietary *P. pavonica* levels on growth performance and somatic indices of *C. zilli*

Groups / Factor	*Padina pavonica* (g/kg diet)					*P*- value
	(0) (C)	2.0 (G1)	4.0 (G2)	6.0 (G3)	8.0 (G4)	
FW (g.)	10.59±0.61 ^{cd}	8.78±0.16 ^{d}	13.46±0.07 ^{c}	21.62±0.58 ^{a}	18.06±2.23 ^{b}	0.00
TL (cm)	8.91±0.24 ^{c}	8.95±0.22 ^{c}	7.79±0.16 ^{d}	13.21±0.16 ^{a}	10.76±0.54 ^{b}	0.00
St. L (cm)	7.28±0.26 ^{c}	7.01±0.13 ^{c}	7.38±0.05 ^{c}	10.17±0.04 ^{a}	9.05±0.53 ^{b}	0.00
WG (g.)	7.22±0.62 ^{cd}	5.25±0.17 ^{d}	9.85±0.09 ^{c}	28.05±0.56 ^{a}	14.45±2.24 ^{b}	0.00
ADG (g.)	0.16±0.01 ^{cd}	0.12±0.00 ^{d}	0.21±0.00 ^{c}	0.62±0.01 ^{a}	0.32±0.05 ^{b}	0.00
SGR%	2.51±0.14 ^{c}	1.99±0.05 ^{d}	2.92±0.04 ^{bc}	4.84±0.05 ^{a}	3.39±0.33 ^{b}	0.00
Condition factor (K)	1.51±0.09 ^{b}	1.38±0.05 ^{b}	2.94±0.17 ^{a}	1.31±0.06 ^{b}	1.41±0.1 ^{b}	0.00
HIS	0.38±0.04 ^{d}	0.24±0.03 ^{d}	7.99±0.49 ^{c}	20.39±0.11 ^{a}	13.93±2.32 ^{b}	0.00
SSI	0.42±0.06 ^{d}	0.55±0.02 ^{d}	6.79±0.33 ^{c}	25.38±0.29 ^{a}	17.26±0.15 ^{b}	0.00
GSI	26.39±1.99 ^{b}	9.81±0.04 ^{d}	19.15±2.29 ^{c}	25.14±2.37 ^{b}	38.74±0.59 ^{a}	0.00

Note that: a-d means that there was statistically significant difference between values at $P \leq 0.05$ within the same row. (n=10/group) W: weight; TL: total length; St: standard length; WG: weight gain; ADG: average daily gain and SGR: specific growth rate. Hepatosomatic index (HSI), spleenosomatic index (SSI) and gonadosomatic index (GSI). A one-way ANOVA test was conducted by using the SPSS statistical program. Values in the same row with the same superscript are not significantly different ($P > 0.05$).

3. Fish body composition

All fish body composition parameters showed a highly significant difference between the experimental groups ($P \leq 0.05$). Proteins and lipids increased significantly in G4 group, while moister and ash recorded the lowest means in the same group (Table 4).

Table 4. Effect of dietary *P. pavonica* levels on whole body composition (%wet weight basis) of *C. zilli*

Groups / Factor	*Padina pavonica* (g/kg diet)					*P*- value
	(0) Control	2.0 (G1)	4.0 (G2)	6.0 (G3)	8.0 (G4)	
Protein	15.19±0.41 ^{c}	15.83±0.42 ^{bc}	16.42±0.31 ^{ab}	16.21±0.38 ^{abc}	17.2±0.21 ^{a}	0.00
Lipid	1.65±0.07 ^{c}	1.76±0.07 ^{c}	1.92±0.1 ^{c}	2.21±0.11 ^{b}	2.9±0.08 ^{a}	0.00
Moister	76.76±0.31^{a}	76.42±0.45 ^{a}	75.28±0.42 ^{bc}	76.29±0.21 ^{ab}	74.98±0.36 ^{c}	0.00
Ash	0.98±0.05 ^{b}	1.26±0.08 ^{a}	1.18±0.09 ^{a}	0.99±0.03 ^{b}	0.94±0.03 ^{b}	0.00

Note that: a-c means that there was statistically significant difference between values at *(P*≤ 0.05) within the same row. (n=10/group). A one-way ANOVA test was conducted by using the SPSS statistical program. Values in the same row with the same superscript are not significantly different (*P > 0.05*).

4. Hematological parameters

All hematological parameters showed a highly significant difference (*P ≤0.05*) within groups. G3 group recorded the highly significant means in all parameters, except for TLC and lymphocytes; whereas, the highly significant means were recorded in G4 group for all parameters (Table 5).

Table 5. Effect of dietary *P. pavonica* levels on hematological parameters of *C. zilli*

Groups / Factor	*Padina pavonica* (g/kg diet)					*P*- value
	(0) Control	2.0 (G1)	4.0 (G2)	6.0 (G3)	8.0 (G4)	
Hb (g/dl)	5.87±0.12 ^{d}	4.25±0.06 ^{e}	6.77±0.13 ^{c}	8.36±0.24^{a}	7.5±0.06 ^{b}	0.00
RBCs (mm^3)	1.13±0.01 ^{c}	0.91±0.03 ^{d}	1.38±0.07 ^{b}	1.53±0.06 ^{a}	1.47±0.01 ^{ab}	0.00
Hct (%)	19.8±0.42 ^{d}	14.37±0.24 ^{e}	21.53±0.45 ^{c}	28.54±0.34 ^{a}	25.09±0.41 ^{b}	0.00
MCV (μm^3)	177.56±0.58 ^{ab}	175.78±0.79 ^{b}	159.63±0.54 ^{d}	179.87±0.81 ^{a}	168.92±1.97 ^{c}	0.00
MCH (g/dl)	52.37±0.47 ^{ab}	51.26±0.34 ^{b}	51.31±0.37 ^{b}	52.82±0.52 ^{a}	51.42±0.48 ^{b}	0.05
MCHC (g/dl)	29.15±0.4 ^{b}	29.31±0.54 ^{b}	22.75±0.48 ^{c}	32±0.37 ^{a}	30.95±0.34 ^{a}	0.00
TLC (µl)	83.59±0.39 ^{c}	72.75±0.57 ^{d}	84.4±0.4 ^{c}	94±0.42 ^{b}	123.85±0.39 ^{a}	0.00
Neutrophil (%)	50.1±0.38 ^{b}	41.45±0.46 ^{c}	36.7±0.39 ^{d}	52.8±0.51 ^{a}	34.3±0.52 ^{e}	0.00
Lymphocyte (%)	38.5±0.51 ^{c}	36.7±0.52 ^{d}	50.1±0.43 ^{b}	50.5±0.45 ^{b}	55.2±0.55 ^{a}	0.00
Monocyte (%)	7.9±0.35 ^{b}	8.6±0.34 ^{b}	10.2±0.39 ^{a}	9.8±0.36 ^{a}	10.2±0.36 ^{a}	0.00
Eosinophil (%)	0.7±0.15 ^{b}	0.7±0.15 ^{b}	1.6±0.16 ^{a}	1.5±0.17 ^{a}	0.7±0.15 ^{b}	0.00
PL (mcL)	34±0.37 ^{c}	32.5±0.48 ^{c}	103.1±0.94 ^{b}	130.1±0.41 ^{a}	130.9±1.32 ^{a}	0.00

Note that: a-e means there were statistically significant difference within values *at (P*≤ 0.05) at the same row. (n=10/group). Hb: hemoglobin; RBCs: red blood cells; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; TLC: total leukocyte count and PL: platelets. A one-way ANOVA test was conducted by using the SPSS statistical program. Values in the same row with the same superscript are not significantly different (*P > 0.05*).

5. Biochemical parameters

All biochemical parameters showed highly significant difference between groups (*P ≤0.05*). Ca, TP, ALB and GL parameters recorded highly significant means in G3 group, while Cr, U and UA means recorded the highly significant means in control group. ALT and AST liver enzymes recorded highly significant difference in G3 group (Table 6).

Table 6. Effect of dietary *P. pavonica* levels on biochemical parameters of *C. zilli*

Groups / Factor	*Padina pavonica* (g/kg diet)					*P. Value*
	(0) Control	2.0 (G1)	4.0 (G2)	6.0 (G3)	8.0 (G4)	
Ca (g/dl)	4.06±0.06 [d]	4.08±0.03 [d]	4.91±0.09 [c]	6.04±0.17 [a]	5.46±0.05 [b]	0.00
TP (g/dl)	4.79±0.09 [c]	5.55±0.06 [b]	1.97±0.02 [d]	6.93±0.08 [a]	5.39±0.08 [b]	0.00
ALB (g/dl)	3.69±0.05 [d]	4.44±0.05 [b]	1.07±0.05 [e]	4.79±0.06 [a]	4.23±0.07 [c]	0.00
GL (g/dl)	1.07±0.02 [d]	1.2±0.05 [c]	0.86±0.01 [e]	2.16±0.04 [a]	1.52±0.04 [b]	0.00
Cr (g/dl)	0.87±0.03 [a]	0.75±0.04 [b]	0.85±0.02 [a]	0.13±0.01 [d]	0.42±0.02 [c]	0.00
U (g/dl)	26.66±0.31 [a]	17.92±0.08 [b]	16±0.09 [c]	15.65±0.09 [c]	13.6±0.09 [d]	0.00
UA (g/dl)	6.72±0.05 [a]	6.44±0.06 [b]	5.27±0.05 [d]	5.67±0.09 [c]	4.71±0.07 [e]	0.00
ALT (U/L)	28.4±0.48 [c]	19.75±0.08 [d]	17.73±0.06 [e]	70.6±0.45 [a]	37.3±0.54 [b]	0.00
AST (U/L)	27.1±0.43 [c]	22.8±0.42 [d]	16.89±0.06 [e]	68.2±0.42 [a]	41.3±0.59 [b]	0.00

Note that: a-e means there was statistically significant difference between values *at (P*≤ 0.05) within the same row. (n=10/group) Ca: Calcium, TP: total protein, ALB: albumin, GL: globulin, CR: creatinine, U: urea, UA: uric acid, ALT: alanine amino-transferase, AST: aspartate amino-transferase. A one-way ANOVA test was conducted by using the SPSS statistical program. Values in the same row with the same superscript are not significantly different (*P> 0.05*).

6. Oxidative stress bio-marker and antioxidant enzymes

MDA, SOD and GSH revealed that highly significant difference (*P≤ 0.05*), while CAT enzyme showed no significant difference between groups (*P> 0.05*). MDA was significantly increased in control group, while SOD and GSH significantly increased in G4 and G3, respectively (Table7).

Table 7. Effect of dietary *P. pavonica* levels on oxidative biomarkers and antioxidant enzymes in *C. zilli*

Groups / Factor	*Padina pavonica* (g/kg diet)					*P. Value*
	(0) Control	2.0 (G1)	4.0 (G2)	6.0 (G3)	8.0 (G4)	
MDA nmol/g. tissue	698.5±0.85 [a]	599.4±0.85 [d]	648.8±0.83 [b]	619±0.65 [c]	319.6±0.54 [e]	0.00
SOD U/g. tissue	7997±1.16 [c]	7997.4±1.19 [c]	6997.4±1.59 [d]	8987.1±1.04 [b]	9994.2±0.81 [a]	0.00
GSH mg. /g. tissue	20.5±0.48 [c]	20.8±0.45 [c]	18.5±0.48 [d]	33±0.52 [a]	23±0.56 [b]	0.00
CAT (U/g. tissue)	19.5±0.45 [a]	19.9±0.41 [a]	19.3±0.52 [a]	18.4±0.45 [a]	18.5±0.48 [a]	0.12

Note that: a-e means there was statistically significant difference between values *at (P*≤ 0.05) within the same row. (n=10/group). A one-way ANOVA test was conducted by using the SPSS statistical program. Values in the same row with the same superscript are not significantly different (*P > 0.05*).

DISCUSSION

Seaweeds are a group of algae with a high content of active compounds, which act as growth promotor and immune enhancer **(Sharawy *et al.*, 2020)**. The fatty-acid compositions of the total lipid contents, determined by capillary gas chromatography, are presented in Fig. (1) and Table (1); the total lipid contents of *P. pavonica* is measured according to **Maghawri *et al.* (2023)**. Palmitic acid (C16:0) was the most abundant constituent between the SFAs in the present study, as indicated from (Table 1). This finding agrees with that of **Gerasimenko and Logvinov (2016)**, who found that palmitic acid is the highest content of SFAs in brown algae, *Padina pavonica* and *Sargassum pallidum*. Five monounsaturated fatty acids (MUSFA) were investigated in the studied species. Oleic acid C18:1(ω9) was the most dominant monounsaturated fatty acid (MUSFA), and this result coincides with that of **Fatma *et al.* (2015)** who observed that oleic acid presented the greatest fraction of the MUSFA in *P. pavonica*. It is noteworthy that, medium quantities of 2-Oleoyl Glycerol C21:1(ω9) was observed as MUSFA. Additionally, three polyunsaturated essential fatty acids (PUSFA) were recorded in *P. pavonica*. Linolic acid C18:2(ω9) recorded the highest content of PUSFA, and this result matches with the findings in the study of **El-Sheekh *et al.* (2021)**. Additionally, 2-Oleoyl Glycerol C21:2(ω9) was detected with a notable concentration of PUSFA group. Generally, fatty acids act as enhancers for several biologically active molecules, such as growth regulators, enzymes and hormones, which exhibit hormonal and immunological activity in fish bodies **(Nahavandi *et al.*, 2023)**.

The present trial recorded a significant increase in growth performance parameters in G3 (6.0g/kg), compared to other groups; this in return concurs with the finding of

Abdelrhman *et al.* (2022), who recorded a significant increase in the feed efficiency of tilapia engaged with increasing brown macroalga (*Sargassum dentifolium*) levels in the diet, compared to the control due to presence of bioactive phytochemical molecules presented in seaweed extract. In this context, **Sharma *et al.* (2014)** reported that diet containing seaweed improves growth and feed efficiency. Moreover, after fed diets supplemented with 0.4% of a brown algae, an enhanced performance was detected in rainbow trout **(Ramalho *et al.*, 2017)**. Conversely, some researchers found that there was no effect of algae on growth performance or feed intake, as elucidated in the study of **Silva-Brito *et al.* (2020)** on the effect of red seaweeds (*Gracilaria* sp.) on gilthead seabream.

In the previous results, HSI and SSI recorded a highly significant difference among groups *($P \leq 0.05$)*, with a specific increase in G3 group. The increase of HSI may be traced back to hyperplasia or hypertrophy of liver cells **(Ayoola, 2008)**, while the increasing SSI is an indicator of a positive effect of algal additive on the spleen as an immune organ reacting for defense. These results coincide with those of **El-daim *et al.* (2021)**, who recorded a significant increase in HSI and SSI in *Oreochromis niloticus* fed with two groups of *Spirulina platensis* (1%) and *Azolla nilotica* (5%) algae. GSI recorded a highly significant difference among groups, with a maximum increase in G4; this increase indicates the high efficiency of algal meal affecting productivity and gonads' quality. This results agrees with that of **Abdulrahman and Hamad (2013)**, who reported a significant increase in GSI of common carp *Cyprinus carpio L.* fed 10% *Spirulina* sp. On the other hand, **Abdulrahman *et al.* (2019)** found no significant effect of *Spirulina* sp. (3 and 5 g/kg feed) on common carp (*Cyprinus carpio L).*

Proximate fish body composition means the calculations of the protein, lipid, moisture and ash content **(Love, 1970)**, which is considered as a good indicator of its physiological processes and health **(Saliu *et al.*, 2007)**. This study recorded that, protein and lipid levels were significantly increased upon using high algal concentrations (G4), which indicated a direct effect of high algal meal with *P. pavonica* on flesh formation. This outcome partly agrees with that of **Younis *et al.* (2018)**, who reported a significant increase in protein levels of *O. niloticus* after fed less than 20% red alga *Gracilaria* sp.; however, no effect was observed on lipid concentrations in the same treatment. In contrary, lipid content of black sea bream, *A. schlegelii*, has shown a decrease upon feeding on a *G. lemaneiformis* based diet with level of 20% **(Xuan *et al.*, 2013)**. Moisture and ash levels decreased significantly with high algal meals using *P. pavonica* (G3 and G4), that's because of the quality of fish productivity which directed the meals to muscles as a proteins and suitable lipids concentrations. In this context, the current finding disagrees with what was stated in the study of **Younis *et al.* (2018)**, who marked high levels of moisture and ash after feeding the Nile tilapia *O.niloticus* a fish meal containing red algae, *Gracilaria arcuata* with different concentrations (20, 40, 60%).

All hematological parameters significantly increased in G3 group, except TLC and lymphocytes in G4 group; that means the algal meal had a positive impact on the health status of *C. zilli* fish in this experiment. **Svobodová *et al.* (2005)** postulated that hematological measures are used to evaluate the health status and feed composition in relation to the habitat of the fish. Usually, WBCs, RBCs and Hct are used for evaluating feed toxicity and fish health status **(Ozovehe, 2013)**.

The results agree with those of **Sattanathan *et al.* (2023)**, who reported an enhancement of hematological parameters of *Labeo rohita* after being fed a mixed algal meal (*Chlorella vulgaris*, *Euglena viridis*, and *Spirulina platensis*). Contrary to our results, **Acar (2018)** stated that feeding common carp on diets supplied with essential oil of H. perforatum for 60 days showed no significant differences in RBCs, Hb%, and Hct values compared to the control.

In addition, the biochemical parameters showed a highly significant difference among all groups, while Ca, TP, ALB, GL and liver enzymes (ALT and AST) increased significantly in G3 group. This result coincides with that of **Mohammadi *et al.* (2020)**, who found similar increases in total protein and albumin levels for the Nile tilapia (*O.niloticus*) after fed extracts of Oregano (*Origanum vulgare*), St John's-wort (*Hypericum perforatum*), and lemon balm (*Melissa officinalis*), reflecting the stronger innate immune response of fish. In this respect, **Jyotirmayee (2015)** reported a significantly increase in TP, ALB and liver enzymes (ALT and AST) of *Labeo rohita* fingerlings after being fed diets supplemented with *Chlorella vulgaris*. While, Cr, U and UA showed a significant decrease in groups fed *P. pavonica* (G3 and G4), and simultaneously an increase was detcted in the control group, indicating the positive effect of the algal meal on waste products as a result of highly body metabolism.

SOD, CAT, and GSH are considered as important enzymes of fish enzymatic system to decrease the oxidative stress **(Adeshina *et al.*, 2021)**. These enzymes could maintain the normal redox homeostasis and improve the normalization in reactive oxygen species **(Abdel-Daim *et al.*, 2019)**. MDA was significantly increased in control group, while the highest activities of SOD and GSH were significantly increased in G4 and G3, respectively. Remarkably, CAT was non significantly affected by different algal concentrations ($P>0.05$). These influences may be attributed to many secondary metabolites and bioactive compounds of *P. pavonica*, which have antioxidant activities affecting fish **(Arunkumar *et al.*, 2021)**. Similar results were reported by **Mohammadi *et al.* (2020)**, who found that hepatic SOD, CAT, and GSH-Px activities were significantly increased with a significant decrease of MDA levels in all fish groups fed on the phytogenic diets when compared with the controls.

CONCLUSION

To conclude, using brown algae (*Padina pavonica*) as a feed additive will help aquaculture farmers, specially those concerned with *Coptodon zilli* farms, to get the

maximum benefits for fish health with low costs. Thus, it is recommended to use *P. pavonica* alga as a feed additive with concentrations of 6.0 and 8.0g/ kg in aquacultures.

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