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Research Article

Partial fishmeal replacement with Spirulina (*Arthrospira platensis*) meal improved growth performance and health indices in Asian seabass (*Lates calcarifer*) juveniles under high-salinity conditions

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Abstract

The effect of fishmeal (FM) replacement with Spirulina (*Arthrospira platensis*) meal (SPM) was investigated in Asian seabass (*Lates calcarifer*) diet. Dietary FM was replaced with SPM at 5 (SP5%) and 10% (SP10%) and a diet without SPM served as the control group. One hundred and thirty five *L. calcarifer* juveniles (45.0 ± 0.2 g) were randomly distributed into nine 300-L circular polyethylene tanks (15 fish/tank). SP10% group had the highest final weight that was associated with better feed conversion ratio value. A higher gut amylase activity was observed in SPM-treated groups than the control group. SP5% group had a higher gut trypsin activity than other treatments. The liver superoxide dismutase activity in SP5% was higher than the other groups. The liver reduced glutathione level increased with increasing SPM level in the diets. The plasma lysozyme activity in SP10% group was higher than other groups. The plasma cholesterol and alkaline phosphatase contents decreased in the fish fed with SP5%. The plasma aspartate aminotransferase decreased in the fish fed with SPM-supplemented diets. Based on the above-mentioned findings, dietary FM replacement with SPM at 10% is recommended for growth and improving health status in *L. calcarifer* juveniles.

Introduction

Finding alternative protein and lipid resources for fishmeal (FM) and fish oil (FO) in aquafeeds is considered as one of the main priorities in aquaculture nutrition research. In this regard, increasing demand for FM and FO, especially for carnivorous fish species, along with the overexploitation of pelagic fish stocks have increased these marine-derived sources prices (Turchini *et al.*, 2019; Kok *et al.*, 2020; Naylor *et al.*, 2021; Rombenso *et al.*, 2022; Macusi *et al.*, 2023). Thus, lowering the amount of FM and FO proportions in the aquafeeds not only can mitigate the pressure on global marine resource but also it can reduce aquafeed production costs and help the sustainability of aquaculture industry (Turchini *et al.*, 2019; Kok *et al.*, 2020; Naylor *et al.*, 2021; Rombenso *et al.*, 2022; Macusi *et al.*, 2023). In the past decade, various novel alternative protein sources for FM were evaluated in aquafeeds, such as single-cell proteins of yeasts, bacteria and microalgae (Hauptman *et al.*, 2014; Pakravan *et al.*, 2017).

Among these single-cell proteins, microalgae is of particular interest because of its exponential growth and high biomass production with low environmental footprint (Hull-Cantillo *et al.*, 2022). Microalgae production does not require arable land and even they can be cultured in unconventional water resources, including agricultural wastewater. Microalgae based on their species contain high amount and well-balanced amino acids profile protein (50–70%), all essential amino acids (EAA), and rich in long chain polyunsaturated fatty acids (LC-PUFA). They also contain bioactive substances with certain nutritional or pharmacological effects, non-digestible polysaccharides, antioxidant compounds, pigments, carotenoids, vitamins, trace elements, and β-glucan (Swanson *et al.*, 2012; Shekarabi *et al.*, 2019; Valente *et al.*, 2021; Saadaoui *et al.*, 2021). These bioactive compound in microalgae can boost up immunocompetence, bactericidal activity, disease resistance and antioxidant capacity of farmed aquatic animals (Reyes-Becerril *et al.*, 2013). Among various microalgae species, Spirulina (*Arthrospira platensis*) (SP), is a preferable candidate to dietary FM replacement because of its high protein content, EAA, high vitamin content (*e.g* E, B1, B5, and B6), particularly vitamin B_{12}, provitamins, minerals (*e.g*. Zn, Mn, Cu, and Se), and many pigments (*e.g.* chlorophyll, β-carotene, and zeaxanthin) (Habib *et al.*, 2008; Kaur *et al.*, 2012). This filamentous blue-green alga contains various bioactive compounds, including carotenoids, phenolic substances, phycobiliproteins (*e.g*. phycocyanin), and polyunsaturated fatty acids such as γ Linolenic acid with antioxidant and anti-inflammatory properties (Hirata *et al.*, 2000; Zhang *et al.*, 2020; Alagawany *et al.*, 2021; Li *et al.*, 2022). Furthermore, SP does not have cellulose cell wall, thus has high digestibility (Karkos *et al.*, 2008). This single-cell protein can be partially replaced with FM in diet for various fish species such as Siberian sturgeon (*Acipenser baeri*, Palmegiano *et al.*, 2005, 2008), rainbow trout (*Oncorhynchus mykiss*, Ahmadzade-Nia *et al.*, 2011), and mullet (*Mugil liza*, Rosas *et al.*, 2019a, 2019b). However, compared to FM, some nutrients such as phosphorus and lysine are low in SPM (Hussein *et al.*, 2014; Macias-Sancho *et al.*, 2014). It has been proved that total dietary FM replacement with SPM create some EAA deficiencies, mainly in the

proportions of lysine, methionine, histidine, arginine and threonine (Rosas *et al.*, 2019a). Thus, more studies are required to optimize the dietary FM replacement with SPM in various farmed aquatic species.

Asian seabass (*Lates calcarifer*) is considered as leading marine fish species for expanding mariculture activity in south of Iran mainly in Persian Gulf and Oman Sea (Mozanzadeh *et al.*, 2020). Previous study in this species proved that dietary FM replacement with SPM did not affect growth performance of this species but replacement levels over 10% reduced blood hemoglobin, hematocrit and enhanced serum aspartate aminotransferase level (Yong *et al.*, 2017). However, this study was conducted in the freshwater and authors did not evaluate other physiological responses such as digestive function in this species. Hence, the present research aimed to assess the impact of replacement of dietary FM with SP meal on performance of *L. calcarifer* reared in sea water.

Materials and methods

The husbandry and treatment of the experimental fish complied with the guidelines of ARRIVE (https://arriveguidelines.org/) and the ethical recommendations guide for using laboratory animals.

Experimental diets

Spirulina meal (65% crude protein, 3.5% crude lipid, 10.1% crude ash) was purchased from Algotab Company (Markazi, Delijan Iran). Spirulina meal was replaced with fishmeal (61.8% crude protein) at 5 (SP5%) and 10 (SP10%) and a control diet did not contain SPM (Table 1).

Table 1: Ingredients and chemical composition (% of dry matter) of experimental diets containing Spirulina.

Ingredients (g/kg diet)[a]	Experimental Diets		
	Control	SP5%	SP10%
Fishmeal	460	410	360
Wheat gluten meal	121	121	121
Corn gluten meal	121	121	121
Soybean meal[b]	50	46	42
Beef gelatin	20	20	20
Spirulina powder	-	50	100
Wheat middling	135	109	83
Fish oil	20	20	20
Canola oil[c]	20	20	20
Soy lecithin[c]	20	20	20
DL-methionine	1	1	1
L-lysine	2	2	2
Vitamin premix[d]	10	10	10
Mineral premix[e]	10	10	10
Butyric acid[f]	2.5	2.5	2.5
Sodium diformate[g]	2.5	2.5	2.5
Vitamin C[h]	5	5	5
Total	1000	1000	1000
Chemical composition			
Dry matter	90.5	90.9	90.7
Crude protein	46.3	45.8	46.7
Crude lipid	16.3	15.1	16.5
Ash	9.1	9.5	9.8

[a] Composition of ingredients as % Dry-weight basis [fishmeal (60.5% crude protein, 18.0% crude lipid); corn gluten (71.4% crude protein, 4.1% crude lipid); wheat gluten (53.3% crude protein, 2.8% crude lipid); soybean meal (41.0% crude protein, 4.2% crude lipid); gelatin (85.0% crude protein, 4.2% crude lipid); *Gracilaria* powder (11.9% crude protein; 1.4% crude lipid); *Sargassum* powder (9.8% crude protein; 1.4% crude lipid); wheat middling (12.0% crude protein, 3.0% crude lipid)].

[b] Product of Kesht Va Sanat Shomal Vegetable Oil Factories Complex (Neca, Iran).

[c] Behpak industrial company, Behshahr, Mazandaran, Iran.

[d] Vitamin premix (IU/kg of premix): Ascorbic acid, 350000; retinol, 1000000000; cholecalciferol, 500000000; tocopherols, 500000; vitamin K_3, 960000; thiamine, 980000; riboflavin, 800000; pyridoxine, 990,000; folic acid, 950000; cobalamin, 10000; biotin, 20000; niacin, 995000; pantothenic acid, 980,000.

[e] Mineral premix (mg/kg of premix): Magnesium, 6,400; copper, 2000; ferrous, 11,000; zinc, 7,000; selenium, 100; iodine, 300; cobalt, 50; natrium, 5,000. ATA Company, Tabriz, Iran.

[f] Merck, Germany.

[g] Conjugated salt of formic acid, HCOOH··HCOO-Na, contained 195 g/kg sodium + 390 g/kg formic acid + 381 g/kg formate + 34 g/kg silicate and water; Formi® NaDF, Addcon Nordic AS.

[h] Rooyan Darou, Semnan, Iran.

In summary, dry ingredients were blended (20 min), then a mixture of soy lecithin and oils were added and mixed (10 min), and finally gelatin dissolved in warm water and was added to prepare a dough. A meat grinder (3 mm) was used to pellet the dough. The pellets were dried (25 ºC, 48 h), then packed and stored (-20°C) until use. The biochemical profile of the all protein sources and the feeds were analyzed based on the standard methods (AOAC, 2000) (Table 2).

Table 2: Growth performance of *L. calcarifer* juveniles fed different experimental diets for 63 days. Data are presented as the mean±pooled SE of three replicates.

Treatments	Control	SP5%	SP10%
IBW (g)[1]	45.4 ± 0.2	44.5 ± 0.4	44.3 ± 0.1
FBW (g)[2]	116.3 ± 0.2[b]	117.5 ± 1.4[b]	130.7 ± 0.8[a]
FBL (cm)[3]	22.3 ± 0.4	22.1 ± 0.3	22.9 ± 0.4
SGR (% / day)[4]	1.5 ± 0.1[b]	1.5 ± 0.1[b]	1.7 ± 0.1[a]
WG (%)[5]	156.4 ±7.5[b]	164.1 ± 8.5[b]	195.5 ± 9.8[a]
FI (g / fish)[6]	75.6 ± 2.0	77.8 ± 2.5	77.8 ± 2.7
FCR[7]	1.1 ± 0.1[b]	1.1 ± 0.1[b]	0.9 ± 0.1[a]
K (%)[8]	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0
HSI (%)[9]	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
VSI (%)[10]	8.6 ± 0.2	8.2 ± 0.1	8.6 ± 0.4
Survival (%)[11]	100 ± 0.0	100 ± 0.0	100 ± 0.0

[1] IBW: initial body weight
[2]FBW: final body weight
[3]FBL: initial body length
[4]SGR: specific growth rate = ((Ln (FBW)-Ln (IBW)) / feeding period (63days)) × 100
[5] WG: weight gain = (FBW (g) – IBW (g) / IBW (g)) × 100
[6] FI: feed intake = total feed intake (g) / number of fish
[7]FCR: feed conversion ratio = FI (g) / WG (g)
[8] K: Fulton's condition factor = FBW (g) / L^3 × 100
[9]HSI: hepatosomatic index = liver weight (g) / body weight (g) × 100
[10] VSI: viscerosomatic index = visceral weight (g) / body weight (g) × 100
[11]SUR: survival = final number of fish / initial number of fish × 100

Fish husbandry

The present study was carried out in Imam Khomeini Marine Fish Research Station, Sarbandar, Khuzestan, Iran. One hundred and thirty five *L. calcarifer* juveniles were randomly distributed into nine 300-L circular polyethylene tanks (15 fish/tank), contained 200 L sand filtered and disinfected (10 ppm calcium chloride, neutralized with 5 ppm sodium thiosulphate) seawater with 80% daily water exchange rate. Each dietary treatment was replicated in triplicate. Feeding was thrice daily (08:00, 12:00 and 16:00) up to satiation, ensuring no pellet was left uneaten for 60 days. Temperature (31.5±2.5°C), salinity (46.0±0.2 g L^{-1}), pH (8.2±0.2) and dissolved oxygen (5.5±0.5 mg L^{-1}) was monitored two time a week. The photoperiod was 12 h light 12 h dark.

Sampling

Fish were unfed 24 h before sampling. Weight and length of fish were individually measured. Six fish of each tank were anesthetized (2-phenoxyethanol, 300 µL L^{-1}) and blood was caught from the caudal vein with heparinized syringes. To measure

complete blood count, an aliquot of the blood (500 µL) was transferred into 1 mL micro-tube and kept in a refrigerator (4°C). To measure plasma biochemical parameters, another aliquot (1000 µL) of blood was centrifuged (5000 *g*, 4°C for 10 min) and the plasma was kept in a freezer (-20 °C). The gut and liver of the same anaesthetized fish were dissected on a piece of ice and transferred into 2 mL microtubes, then kept in a freezer (-80°C).

Digestive enzymes and antioxidant factors

The gut samples were homogenized in ice-cold mannitol buffer (50 mM manitol + 2 mM tris-HCl, pH 7) (30:1; v/w) for 60 s (Castro-Ruiz *et al.,* 2019). The homogenate was centrifuged, then the supernatant was separated (10min, 9000 ×*g*, 4°C). For separating the gut's brush border, the supernatant was centrifuged (34,000 × g, 10 min, 4°C) and the precipitate was dissolved in one mL of buffer (0.1 M KCl, 5 mM Tris-Hepes, 1 mM DTT; pH 7.5) to evaluate alkaline phosphatase (ALP) (Crane *et al.,* 1979; Gisbert *et al.,* 2019). Soluble protein content (Bradford, 1976), trypsin (Bergmeyer, 1974), chymotrypsin (Hummel, 1959), protease (Folin and Ciocalteau, 1929), ALP (Bessey *et al.,* 1946), α-amylase (Bernfeld, 1955), and lipase (Tietz and Fiereck, 1966) activities were measured based on standard methods. The liver samples were homogenized (1: 9, w/v) in cold potassium phosphate buffer (0.1 M, pH=7.4, 4°C, 10000 ×*g*) for 60 s. The homogenate was centrifuged (9,000 *g*, 30 min, 4°C); the supernatant was separated to evaluate antioxidant parameters. Catalase (CAT) (Aebi, 1974), superoxide dismutase (SOD) (McCord and Fridovich, 1969) and glutathione level (GSH) (Beutler *et al.,* 1963) were measured based on standard protocols. Soluble protein levels were measured using Bradford (1976) method (Table 3).

Table 3: Digestive and liver antioxidant enzymes activity of *L. calcarifer* juveniles fed different experimental diets for 63 days. Data are presented as the mean ± pooled SE of three replicates.

Treatments	Control	SP5%	SP10%
Gut digestive enzymes			
Amylase (U / mg protein)	0.3 ± 0.1^{b}	1.5 ± 0.3^{a}	1.3 ± 0.2^{a}
Lipase (U / mg protein)	402.2 ± 46.4^{a}	434.3 ± 16.8^{a}	259.1 ± 28.3^{b}
Alkaline phosphatase (U / mg protein)	6.3 ± 0.7	7.4 ± 1.3	6.5 ± 0.5
Total protease (U / mg protein)	1158.3 ± 120.6	1029.4 ± 103.4	823.9 ± 135.9
Trypsin (U / mg protein)	14.3 ± 0.6^{b}	17.9 ± 0.7^{a}	12.6 ± 1.2^{b}
Chymotrypsin (U / mg protein)	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Liver antioxidant parameters			
Catalase (U / mg protein)	227.1 ± 23.5^{a}	203.9 ± 15.3^{a}	139.8 ± 18.8^{b}
Superoxide dismutase (U / mg protein)	19.2 ± 2.8^{b}	29.2 ± 2.6^{a}	22.2 ± 2.1^{b}
Glutathione (µmol / g tissue)	16.5 ± 2.0^{c}	26.7 ± 2.4^{b}	33.3 ± 4.2^{a}

Hematological and plasma biochemical indices

Complete blood count parameters including red blood cell (RBC), white blood cell (WBC), hemoglobin (HB), and hematocrit (Hct) were assessed, as described by Blaxhall and Daisley (1973). Blood indices, including the mean cell hemoglobin (MCH), the mean cell volume (MCV) and the mean cell hemoglobin concentration

(MCHC), were calculated according to Dacie and Lewis (2001). Respiratory burst activity (RBA) (Siwicki *et al.*, 1994) and lysozyme activity (Ellis, 1990) of plasma was determined by standard methods. Plasma biochemical parameters, including glucose (GLU), cholesterol (CHOL), triglycerides (TRIG), total protein (TP), albumin (ALB), calcium (Ca), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were measured spectrophotometrically by applying diagnostic kits (MAN company, Tehran, Iran) and globulin determined by the following formula:

Globulin (GLOB)=TP–ALB.

Statistical analysis

Data were analyzed by SPSS statistics software (V. 23.0, Chicago, IL, USA) and presented as means±standard error (n=3). First, data normality of distribution was evaluated by Kolmogorov-Smirnov test then Levene's test was used to evaluate data homogeneity of variances. A one-way ANOVA followed by Duncan's post test were used to evaluate significant differences at $p<0.05$.

Results

Survival rate was 100% in all treatments. Fish in SP10% group had the highest final weight and specific growth rate. Feed conversion ratio improved in fish fed SP10% diet. Somatic indices, including Fulton's condition factor (K), hepatosomatic index (HSI) and viscerosomatic index (VSI) did not change among groups.

Amylase activity increased in fish fed SPM-included diets ($p<0.05$). SP10% group had lower lipase activity than the other groups. SP5% group had higher trypsin activity than the other treatments. Alkaline phosphatase, total protease and chymotrypsin activities did not affect by experimental diets ($p>0.05$). SP10% group had lower CAT activity than the other groups. Superoxide dismutase activity in SP5% group was higher than the other treatments. Liver glutathione level increased with increasing SPM level in diet.

Wight blood cell count increased in fish fed SP5% diet (Table 4).

Table 4: Hemato-immunological parameters of *L. calcarifer* juveniles fed different experimental diets for 63 days. Data are presented as the mean ± pooled SE of three replicates.

Treatments	Control	SP5%	SP10%
RBC ($\times10^6$/ µL)	2.1 ± 0.4	2.8 ± 0.5	2.5 ± 0.1
WBC ($\times10^3$/ µL)	3.3 ± 0.6^{ab}	4.6 ± 0.5^{a}	3.0 ± 0.1^{b}
HCT (%)	44.0 ± 3.0	41.7 ± 2.1	43.0 ± 3.5
HB (g / dL)	6.4 ± 0.5	6.3 ± 0.4	7.7 ± 0.4
RBA (OD 540)	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
LYZ (U/mL)	770.4 ± 52.1^{b}	663.0 ± 39.9^{b}	1096.2 ± 7.4^{a}

Abbreviations: RBC, red blood cell; WBC, white blood cell; HCT, hematocrit; HB, hemoglobin; RBA, respiratory burst activity; LYZ, lysozyme.

SP10% group had higher lysozyme activity than the other groups ($p> 0.05$). Red blood cell count, HCT, HB, and RBA did not affect by the experimental diets. Plasma CHOL and ALP contents significantly decreased in fish fed SP5% diet (Table 5).

Plasma calcium content increased in fish fed SP10% diet. Plasma AST decreased in fish fed SPM-supplemented diets than the control. Other biochemical parameters, including GLU, TRIG, TP, ALB, GLOB, and ALT did not change among treatments.

Table 5: Plasma biochemical parameters of *L. calcarifer* juveniles fed different experimental diets for 63 days. Data are presented as the mean ± pooled SE of three replicates.

Treatments	Control	SP5%	SP10%
GLU (mg / dL)	84.9 ± 9.5	59.2 ± 8.9	80.6 ± 11.0
CHOL (mg / dL)	151.8 ± 11.7^{a}	118.5 ± 8.5^{b}	139.0 ± 8.7^{ab}
TRIG (mg / dL)	83.1 ± 9.2	72.3 ± 6.0	71.1 ± 12.2
TP (g / dL)	2.8 ± 0.2	2.6 ± 0.2	2.8 ± 0.1
ALB (g / dL)	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
GLOB (g / dL)	1.8 ± 0.2	1.8 ± 0.2	1.9 ± 0.1
Ca (mg / dL)	6.6 ± 0.8^{b}	7.3 ± 1.0^{b}	11.5 ± 1.9^{a}
ALP (U / L)	5.5 ± 0.4^{a}	3.3 ± 0.3^{b}	5.0 ± 0.2^{a}
ALT (U / L)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
AST (U / L)	0.7 ± 0.1^{a}	0.4 ± 0.1^{b}	0.5 ± 0.0^{b}

Abbreviations: GLU, glucose; CHOL, cholesterol; TRIG, triglyceride; TP, total protein; ALB, albumin; GLOB, globulin; Ca, calcium; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Discussion

In this research, the dietary FM replacement with 10% SPM significantly enhanced growth that mainly associated with better FCR value, high amylase activity and increased liver GSH level and plasma lysozyme activity in this species. It has been confirmed that SP by improving gut microbiome balance due to its prebiotic effects could improve nutrients digestibility and absorption, increase digestive enzyme activities and lipid metabolism (Nandeesha *et al.*, 1998; Teimouri *et al.*, 2013, 2016; Adel *et al.*, 2016). Furthermore, spirulina is rich in numerous nutrients, especially vitamins, minerals, nucleotides, carotenoids, EAA, fatty acids and bioactive compounds with antioxidant and immunostimulatory effects that may promote growth and feed utilization in this species (Mustafa *et al.*, 1994; Abdel-Tawwab and Ahmad, 2009). Similarly, Yong *et al*. (2017) demonstrated that the dietary replacement of FM with SP over 10% decreased growth performance in *L. calcarifer* but it was not statically significant due to large standard deviations in the experimental groups, but FCR value increased by 30% FM replacement level. Moreover, Siddik *et al*. (2022) reported that dietary FM replacement with selenium enriched SPM up to 10% did not affect growth performance in *L. calcarifer*, but 20% replacement level adversely affect growth in this species. Previous studies also reported successful partial FM replacement with SPM in various fish species such as silver seabream (*Rhabdosargus sarba*, 50%; El-Sayed, 1994), and mullet (*Mugil liza*, 50%; Rosas *et al.*, 2019a).

In the current study, dietary FM replacement with SPM enhanced amylase activity and at 5% replacement level trypsin activity increased in *L. calcarifer*. It has been suggested that microalgae can stimulate the propagation of beneficial

bacteria in the gut and result in more endogenous digestive enzymes synthesis (Anand *et al.,* 2013; Adel *et al.,* 2016). It has been confirmed that spirulina can improve gut microflora through prebiotic effects, increase digestive enzymes activity that facilitate the digestion of indigestible components. In this context, lipase and protease activities enhanced in great sturgeon fed diets contained 5-10% SPM (Adel *et al.,* 2016). Protease activity increased in Oscar fish fed a diet supplemented with 5.5% SPM (Mohammadiazarm *et al.,* 2021). Supplementing a diet with 10% SPM significantly increased protease, lipase and amylase in Koi (*Cyprinus carpio*, Ansarifard *et al.,* 2018). Moreover, supplementing diet with 0.08% SPM in Yellow River carp (*Cyprinus carpio*) significantly enhanced trypsin, lipase and amylase activities (Ren *et al.,* 2021). Furthermore, inclusion of dietary 5% SPM increased gut protease and lipase activities in juvenile grass carp (*Ctenopharyngodon idella*), meanwhile gut amylase activity increased by graded inclusion of SPM (1-10%) in diet (Faheem *et al.,* 2022).

Our findings showed, dietary FM replacement with 10% SPM reduced lipase activity compared to the control indicating inhibiting effects of SPM at high inclusion level. In this context, it has been observed that the aqueous extract of *Spirulina platensis* containing the non-protein components that inhibit lipase activity in vitro and can prevent the postprandial elevation of blood lipid levels (Kishibuchi *et al.,* 2019) as also was noticed in the present study, especially regarding plasma cholesterol level in fish fed SP10% diet.

In this research, liver GSH level increased with increasing the inclusion of SPM in diet suggesting SPM can enhance antioxidant capacity in *L. calcarifer*. In addition, 5% FM replacement with SPM enhanced SOD activity, but 10% replacement level reduced CAT activity compared to control group in the liver of *L. calcarifer*. Spirulina contains various antioxidants such as phycocyanin, vitamin E and pigments (e.g., β-carotene and xanthophylls phytopigments) (Estrada *et al.,* 2001; Abdel-Tawwab and Ahmad, 2009; Franova *et al.,* 2010). Phycocyanins have scavenging action for quenching reactive oxygen species (i.e. O_2^-, H_2O_2, OH^-) and inhibit lipid peroxidation (Bermejo *et al.,* 2008). In this context, Siddik *et al*. (2022) reported that dietary FM replacement with selenium-enriched SPM significantly enhanced serum GPx activity, but it did not affect CAT in *L. calcarifer*. In juvenile gibel carp (*Carassis auratus* gibelio var. CAS III) dietary FM replacement with SPM increased SOD and total antioxidant capacity (Cao *et al.,* 2018). Also, supplementing 2-4% SPM in diet increased total antioxidant capacity in the liver of mullet (*Mugil liza*) that was attributed to a significant reduction of lipid peroxidation level (Rosas *et al.,* 2019b).

In the present study, replacement of dietary FM with SPM at 5% level significantly increased WBC compared to other groups suggesting immunomostimulatory effects and anti-infection properties of the algal mixture. It has been reported that the presence of phycocyanin in spirulina increased WBC in mice (Zhang, 1994). In addition, polysaccharides (e.g. carrageenan,

alginates, β-glucans, and sodium alginate) in seaweeds have great immunostimulatory effects and could increase diseases resistance in fish (Adel et al., 2016). In other fish species supplementing diet with SPM increased hematological parameters such as WBC count in Labeo rohita fingerlings (Andrews *et al*., 2011), RBC and WBC counts in rainbow trout (Yeganeh *et al.,* 2015), RBC, HB and HCT in great sturgeon (Adel *et al.,* 2016), WBC in common carp (Samah *et al.,* 2017), RBC, WBC and HB in Coral trout (Yu e*t al.,* 2018), RBC, WBC, HCT and HB in Stinging catfish (*Heteropneustes fossilis*, Rahman *et al.,* 2023).

It is reported that SPM can trigger immune responses in fish due to the presence of various bioactive ingredients such as β-carotene, phycocyanin and polysaccharides (Cerezuela *et al.,* 2012). In this research, plasma lysozyme activity increased with dietary SPM inclusion level suggesting immunostimulatory effects of this alternative protein source. Similarly, inclusion of dietary SPM significantly increased lysozyme activity in African sharp tooth catfish (Promya and Chitmanat, 2011), gibel carp (Cao *et al*., 2018a, b), and great sturgeon (Adel *et al.,* 2016), coral trout (Yu *et al.,* 2018), Oscar fish (Mohammadiazarm *et al.*, 2021).

Spirulina contains bioactive compounds (e.g. phycocyanin, β-carotene and phenolic components), with hypocholesterolemia effects (Colla *et al.,* 2008). In this study, plasma CHOL significantly decreased in fish fed SPM supplemented diets, especially in SP5% group suggesting hypolipidemic effect of algal mixture. Similarly, supplementing diet with single or a mixture of *Gracilaria gracilis* and microalga *Nannochloropsis oceanica* significantly reduced serum TRIG in European seabass (Batista *et al.,* 2020). Velasquez *et al.* (2016) reported that supplementing diet with SPM significantly reduced serum TRIG in Nile tilapia.

In our research, plasma calcium increased in SP10% group may due to higher bioavailability of calcium in these ingredients leading to higher their absorption (Xu *et al.,* 2020). In the current research, plasma ALP reduced in fish fed SP5% and plasma AST decreased in fish fed SPM-supplemented diets compared to control that may indicate health promoting effects of SPM on the liver. Also, dietary 0.1% SPM supplementation significantly reduced serum AST in Nile tilapia (Shalata *et al.,* 2021). In addition, 5% dietary FM replacement with selenium enriched SPM significantly reduced serum ALT and AST in *L. calcarifer* (Siddik *et al.,* 2022). However, replacement of dietary FM with SPM over 20% significantly increased serum AST in *L. calcarifer* (t).

Conclusions

The results of the current research showed that dietary FM replacement with SPM at 10% level increased growth performance that associated with better FCR value in *L. calcarifer*. In addition, supplementing diet with SPM increased amylase activity and at 5% replacement level enhanced trypsin activity in fish. The liver GSH level and plasma lysozyme activity increased with increasing the amount of SPM in diet. In addition, at 5% replacement level liver SOD and blood WBC increased suggesting modulatory effects of SPM on antioxidant

capacity and immunocompetance of fish. In addition, SMP showed distinct hypocholestremia effects by lowering plasma CHOL in L. calcarifer. Plasma calcium level increased with the increment of SPM in diet. Finally, plasma AST level decreased by inclusion of SPM in diet suggesting health promoting effects of this alternative protein source on the liver tissue. More studies are required at molecular level such as transcriptomics to finds out the SPM mode of action in promoting performance and health status of fish.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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