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RESEARCH

Potential of *Scenedesmus sp.* and *Aurantiochytrium spp.* as protein and n-3 fatty acid sources in practical diets for juvenile *Penaeus vannamei*

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Abstract

This study investigated the potential of *Scenedesmus* sp. (SCM) and *Aurantiochytrium* spp. (AUM) meals as protein and lipid sources in practical diets for juvenile *Penaeus vannamei*. In phase I, a reference diet (0-R) was formulated with 150.0 g kg^{-1} fish meal (FM). Four other diets replaced FM with SCM at 25, 50, 75, and 100%. Diets were evaluated with 2,000 shrimp of 1.99 ± 0.15 g body weight (BW) stocked in 40 indoor tanks of 0.5 m^3 under 80 animals m^{-2} for 75 days. Subsequently, feed preference was assessed by simultaneously confronting all diets and measuring their relative apparent feed intake (RAFI). In phase II, a control diet was formulated with 120.0 g kg^{-1} FM and 12.0 g kg^{-1} fish oil. Four fish-free diets fully replaced these ingredients with 50.0, 200.0, 250.0, and 300.0 g kg^{-1} SCM and a fixed level of AUM at 15.0 g kg^{-1}. A total of 3,570 shrimp of 1.78 ± 0.16 g were stocked in thirty-five 1-m^3 outdoor tanks under 100 animals m^{-2} and fed for 71 days. In phase I, final shrimp survival (96.6 ± 3.4%), weekly growth (1.09 ± 0.05 g), gained yield (982 ± 47 g m^{-2}), and FCR (1.56 ± 0.07) were unaffected by FM replacement ($P > 0.05$). RAFI significantly dropped at 75 and 100% FM replacements ($P < 0.05$). The highest RAFI was observed for the reference diet and the lowest when FM was completely removed ($P < 0.05$). In phase II, final survival (94.3 ± 3.8%), and weekly growth (0.85 ± 0.02 g) did not differ significantly. Shrimp fed 0-I diet achieved the highest BW (12.24 ± 0.93 g) and those fed diet 300-I the lowest (9.25 ± 1.00 g; $P < 0.05$). The highest gained yield (921 ± 70 and 828 ± 45 g m^{-2}), and the lowest FCR (1.52 ± 0.10 and 1.61 ± 0.08) was found for shrimp raised with the reference and 200-I diets, respectively ($P < 0.05$). Our findings indicate that *Scenedesmus* sp. meal can be included in shrimp diets up to 150 g kg^{-1} and fully replace FM protein on a weight-to-weight basis. However, replacing all marine proteins and oils with *Scenedesmus* sp. and *Aurantiochytrium* spp. meals significantly reduced feed attractability and shrimp performance. Future research should focus on refining fish-free diet formulations containing SCM and AUM to ensure they meet all essential nutrient requirements and improve feed attractability.

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Keywords Fish-free diets · *Scenedesmus* sp. · *Aurantiochytrium* spp. · *Penaeus vannamei* · Microalgae · DHA-algal extract · Fish meal replacement

Introduction

The whiteleg shrimp, *Penaeus vannamei*, a major species in global shrimp farming, has experienced a significant production increase over the past decade, rising from 3.1 million mt. in 2012 to 6.8 million mt. in 2022 (FAO 2024a). The species now accounts for a substantial share of the global crustacean market (FAO 2024b). This growth has increased the need for effective and sustainable feed ingredients, as feed represents a major portion of production costs.

Shrimp have specific nutritional requirements that demand high-quality protein sources. Ideally, these protein sources should offer a high protein content, an appropriate amino acid profile, high digestibility, good palatability, the absence of antinutritional factors, a reliable supply, and economic efficiency (NRC 2011; Vizcaíno et al. 2014). Traditionally, fish meal (FM) and soybean meal (SBM) have been the primary protein sources in shrimp diets due to their excellent nutritional qualities and cost-effectiveness. However, these ingredients can pose sustainability challenges. FM production can contribute to overfishing, potentially impacting wild fish stocks, while soybean cultivation has raised concerns about deforestation, water usage, and the use of GMOs (Genetically-Modified Organisms) and pesticides (Tacon and Metian 2009; Lima et al. 2011). Additionally, the rising cost of FM, driven by increasing demand and limited production, may affect the economic sustainability of shrimp farming operations. Similarly, fish oil, a key source of essential fatty acids, faces sustainability issues. IUU (illegal, unreported and unregulated fishing) can lead to overfishing to obtain fish oil which can deplete marine resources, making it crucial to find alternative sources of long-chain n-3 fatty acids.

To address these sustainability and economic issues, the aquafeed industry is actively seeking alternative protein and lipid sources that can replace FM, SBM, and fish oil without compromising the nutritional quality of the feed. This shift is critical for both environmental sustainability and the economic viability of shrimp farming. Reducing reliance on these traditional ingredients can result in significant savings and lower production costs while mitigating potential environmental impacts.

Microalgae have emerged as a promising alternative for aquafeeds. They are rich in bioactive compounds, with protein content reaching up to 700 g kg^{-1}, and provide a balanced profile of essential amino acids (Huy et al. 2022; Lucakova et al. 2022). Additionally, microalgae are high in long-chain n-3 fatty acids, sulfated carbohydrates, antioxidants, vitamins, minerals, sterols, and carotenoid pigments (Selvan et al. 2024). Their potential for carbon capture in biorefineries further enhances their sustainability (Shitanaka et al. 2024).

Research has demonstrated the successful inclusion of microalgae in aquafeeds for various fish species (Nobrega et al. 2019; Carvalho et al. 2020; Batista et al. 2021). However, their use in shrimp diets remains underexplored. This study aims to investigate the potential of the microalgae species *Scenedesmus sp.* and *Aurantiochytrium spp.* as primary sources of protein and long-chain n-3 fatty acids in diets for juvenile *P. vannamei*. Specifically, the study aimed at determining the optimal replacement levels of traditional FM with *Scenedesmus sp.* and evaluate the combined use of *Scenedesmus sp.* and *Aurantiochytrium spp.* to

develop sustainable, fish-free diets. By exploring these microalgae species, this study seeks to contribute to more sustainable shrimp farming practices, offering viable alternatives to traditional feed ingredients.

Material and methods

Experimental design

The study comprised two separate experiments conducted consecutively in two distinct rearing systems (Table 1). In the initial phase (phase I), the objective was to determine the maximum replacement level of FM with *Scenedesmus* sp. meal (SCM). Five diets were formulated to gradually replace FM with SCM. In the subsequent phase (phase II), shrimp were fed diets devoid of FM and fish oil, incorporating SCM and *Aurantiochytrium* spp. (AUM) as protein and lipid sources, respectively. In this phase, juvenile shrimp were fed with increasing dietary levels of SCM along with a constant level of AUM. At harvest, shrimp were individually counted and weighed to determine their final survival, weekly growth rate, final body weight (BW), gained yield, apparent feed intake (AFI), and feed conversion ratio (FCR). Additionally, a seven-day evaluation of feed intake was conducted with the first set of diets. All diets were delivered simultaneously in five separate feeding trays positioned opposite to each other on the tank bottom near the tank wall. After one hour, feed remains were collected, dried, and weighed to determine the relative apparent feed intake (RAFI, %).

Scenedesmus sp. and Aurantiochytrium spp. meals and diet formulation

SCM was laboratory-produced by Algae Biotecnologia Ltda. (Holambra, Brazil). The ingredient was freeze-dried and contained (%, as-is basis) 559.4 g kg^{-1} crude protein (CP), 56.7 g kg^{-1} ether extract (EE), 82.3 g kg^{-1} crude fiber (FB), 112.5 g kg^{-1} ash, 24.7 g kg^{-1} phosphorus, and 9.2 g kg^{-1} calcium (Table 2). SCM contained lower levels of essential amino acids (EAAs) compared to FM, such as methionine (Met, 9.5 versus 15.6 g kg^{-1}), lysine (Lys, 33.1 vs. 41.5 g kg^{-1}) and methionine plus cysteine (M + C, 15.3 vs. 20.6 g kg^{-1}), respectively. The algal extract consisted of a dried powder from a proprietary

Table 1 Details of the two study phases

Characteristics	Study Phase	
	I	II
Rearing system and tank volume	Indoor, 0.5 m^3	Outdoor, 1.0 m^3
Number of tanks (replicates)	40 (8)	35 (7)
Number of diets	Five with one reference	Four with one control
Initial shrimp body weight	1.99 ± 0.15 g	1.78 ± 0.16 g
Initial stocking density	88 shrimp m^{-2}	100 shrimp m^{-2}
Rearing period	75 days	71 days

Table 2 Proximate and amino acid composition of the microalgae *Scenedesmus* sp. and salmon meal used in the present study

Proximate composition	Composition (g kg^{-1}, as-is basis)	
	Scenedesmus sp.	Salmon meal
Dry matter (DM)	968.4	842.3
Crude protein	559.4	593.8
Ether extract	56.7	94.0
Total fiber	82.3	5.0
Ash	112.5	229.0
Calcium	9.2	67.6
Phosphorous	24.7	36.1
Gross energy (MJ kg^{-1}, DM)[a]	200.3	187.9
Essential amino acids (EAA)		
Arginine	29.0	35.8
Histidine	9.7	15.2
Isoleucine	20.8	22.4
Leucine	45.3	39.8
Lysine	33.1	41.5
Methionine	9.5	15.6
Methionine + Cysteine	15.3	20.6
Phenylalanine	28.4	21.4
Threonine	27.4	24.4
Tryptophan	5.6	8.4
Tyrosine	17.5	31.2
Valine	27.4	26.4
Non-essential amino acids (NEAA)		
Alanine	45.3	39.2
Aspartic acid	54.8	44.4
Cysteine	5.8	5.1
Glycine	31.3	3.8
Glutamic acid	55.9	67.9
Proline	24.6	30.8
Serine	23.3	17.5
Sum EAA	253.7	282.1
Sum NEAA	241.0	208.7
Sum EAA + NEAA	494.7	490.8

[a]Calculated as GE (gross energy) = (4,143 + (560 × ether extract [DM]) + (150 × crude protein [DM]) – (440 × ash [DM])) × 0.0041868

strain (Nymega™, Helia®, Gilbert, USA) of *Aurantiochytrium* (aka *Schizochytrium*) spp. (AUM), reported to contain a minimum of 200 g kg^{-1} DHA.

In study phase I, a reference diet (0-R) was formulated to contain 400.0 g kg^{-1} soybean meal (SBM; % of the diet, on an as-is basis), 150.0 g kg^{-1} FM, 28.5 g kg^{-1} wheat gluten meal, and 10.0 g kg^{-1} soy protein concentrate (SPC; Table 2). From the 0-R diet, four other diets were designed to replace FM with SCM at 25, 50, 75, and 100%. This corresponded to a dietary inclusion of FM and SCM of 112.5 and 37.5 g kg^{-1} (diet 25-R, % replacement), 75.0 and 75.0 g kg^{-1} (50-R), 37.5 and 112.5 g kg^{-1} (75-R), and 0 and 150.0 g kg^{-1}

(100-R), respectively. Dietary crude protein was balanced by increasing the inclusion SPC at the cost of wheat flour. The dietary levels of methionine (Met) and lysine (Lys) were adjusted through supplementation with DL-Methionine and L-Lysine, respectively. Similarly, the inclusion of fish oil and soy lecithin oil increased with higher levels of SCM to meet the required dietary levels of essential long-chain polyunsaturated omega-3 fatty acids (n-3 LC-PUFA) and phospholipids. Diets were also balanced for magnesium, calcium, and potassium. Krill meal and squid meal were included at a fixed level of 10.0 g kg^{-1} each across all diets to stimulate feed attractability and palatability.

In study phase II, a control diet (0-I) was formulated to contain 308.7 g kg^{-1} SBM, 120.0 g kg^{-1} FM, 80.0 g kg^{-1} SPC, 50.0 g kg^{-1} wheat gluten meal, and 12.0 g kg^{-1} fish oil (Table 3). Four other diets consisted solely of plant-derived proteins and lipids. In this case, FM and fish oil were eliminated and replaced with SCM, AUM, and soybean oil. SCM was included at 150.0 (diet 150-I), 200.0 (200-I), 250.0 (250-I), and 300.0 g kg^{-1} (300-I) at the expense of SBM. The dietary inclusion of AUM was fixed at 15.0 g kg^{-1} across all fish-free diets. As in the first set of diets, those used in phase II were also supplemented with crystalline amino acids (CAAs) to balance the dietary levels of Met, Lys, and threonine (Thr).

Diet preparation and chemical analysis

Diets were prepared in the lab following the methodology described by Nunes et al. (2011). Briefly, all dried ingredients were ground to 300 µm, weighed and mixed with the liquid raw materials and feed additives in a planetary mixer until a feed dough was formed. The feed dough was then manually broken into small particles by forcing it though a rigid net. The particles were introduced into a laboratory extruder adjusted to operate at 95°C. After extrusion, pellets of 2.0 mm in diameter by 4.8 mm in length were steamed for 3 min. and then dried in a convection oven until moisture was reduced to 8.99 ± 0.61% (n = 8). Dried pellets were stored under 16°C until use.

Finished diets were chemically analyzed (AOAC 2023). Dry matter (DM) was determined by drying samples in a convection oven for 24 h at 105°C. The Dumas combustion method was applied to analyze crude protein (CP, AOAC 968.06), while ether extract (EE) was determined through acid hydrolysis (AOAC 954.02). Ash content was determined by burning samples in a muffle furnace at 600°C for 2 h (AOAC 942.05) and crude fiber by enzymatic–gravimetric determination (AOAC 992.16). Amino acid (AA) and fatty acid (FA) compositions were determined using high-performance liquid chromatography (White et al. 1986; Hagen et al. 1989) and high-resolution gas chromatography (GC) with a flame ionization detection fitted with a capillary GC column, respectively.

Diets produced for study phase I resulted in a total CP and EE content of 364.5 ± 1.3 g kg^{-1} (mean ± standard deviation, sd) and 92.1 ± 1.8 g kg^{-1} (Table 3), respectively. The dietary AA composition was consistent among diets, except for histidine, aspartic acid, cysteine, and, taurine which showed a decreasing trend with higher levels of FM replacement (Table 4). Dietary Met (M + C) content ranged from 8.6 (11.6) and 9.5 g kg^{-1} (16.5 g kg^{-1}), while Lys varied from 19.7 to 20.4 g kg^{-1}.

For phase II, diets showed a total CP and EE content of 361.1 ± 10.0 and 90.2 ± 4.5 g kg^{-1}, respectively (Table 3). Total dietary Met (M + C), Lys, and Thr reached 8.3 ± 0.4, 21.8 ± 1.5 (12.9 ± 0.4), and 14.5 ± 0.9 g kg^{-1}, respectively (Table 4). The total FA composition varied between the 0-I and fish-free diets (Table 5), particularly regarding the HUFA (highly unsaturated fatty acids) profile. Fish-free diets were completely devoid of

Table 3 Ingredient and proximate composition of experimental diets

Ingredients/Diets[a]	Ingredient Composition (g kg^{-1} of the diet, as-is)									
	Diets/Study Phase I					Diets/Study Phase II				
	0-R	25-R	50-R	75-R	100-R	0-I	150-I	200-I	250-I	300-I
Soybean meal[b]	400.0	400.0	400.0	400.0	400.0	308.7	300.0	238.0	178.0	118.0
Wheat flour[c]	231.5	218.4	206.3	200.0	180.0	285.2	242.1	257.9	265.2	272.6
Salmon meal[d]	150.0	112.5	75.0	37.5	-	120.0	-	-	-	-
Scenedesmus sp.[e]	-	37.5	75.0	112.5	150.0	-	150.0	200.0	250.0	300.0
Soy protein concentrate[f]	10.0	18.0	25.1	31.7	40.8	80.0	80.0	80.0	80.0	80.0
Wheat gluten meal[g]	28.5	28.5	28.5	28.5	28.5	50.0	50.0	50.0	50.0	50.0
Salmon oil	368	36.4	35.5	34.4	33.8	12.0	-	-	-	-
Soy lecithin oil	2.7.2	29.8	32.4	35.1	37.5	29.7	37.9	37.9	37.9	37.8
Soybean oil[h]	-	-	-	-	-	20.0	16.7	16.2	15.5	14.8
Aurantiochytrium spp.[i]	-	-	-	-	-	-	15.0	15.0	15.0	15.0
Magnesium sulfate	25.8	27.5	29.3	30.0	30.0	25.3	27.5	29.4	31.3	33.3
Calcium carbonate	18.0	18.0	18.0	18.0	18.0	16.9	25.0	18.5	18.0	17.4
Potassium chloride	13.9	14.9	16.0	13.2	18.1	-	0.1	2.0	4.4	6.8
Salt	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Krill meal[j]	10.0	10.0	10.0	10.0	10.0	-	-	-	-	-
Squid meal[k]	10.0	10.0	10.0	10.0	10.0	-	-	-	-	-
Vitamin-mineral premix[l]	10.0	10.0	10.0	10.0	10.0	8.0	8.0	8.0	8.0	8.0
Dextrin	5.0	5.0	5.0	5.0	9.3	-	-	-	-	-
Synthetic binder[m]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DL-Methionine[n]	3.0	3.3	3.5	3.8	4.0	2.2	3.5	3.6	3.6	3.6
L-Lysine[o]	-	0.2	1.0	1.5	1.8	3.1	7.3	8.1	8.7	9.4
L-Threonine[p]	-	-	-	-	-	1.3	2.2	2.4	2.6	2.8
Choline chloride[q]	-	-	-	-	-	1.8	1.8	2.1	2.5	2.8
Cholesterol[r]	0.1	0.3	0.5	0.6	0.6	1.0	1.0	1.0	1.0	1.0
Vitamin C[s]	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5	0.5
Monosodium phosphate	5.0	4.3	3.7	3.0	2.4	19.6	16.2	14.5	12.8	11.1
Proximate composition (g kg^{-1}, as-is)										
Dry matter	908.2	903.7	906.9	901.8	922.4	914.7	908.3	911.3	912.3	900.6
Crude protein	363.1	363.5	365.2	366.4	364.2	351.7	354.7	356.3	367.0	375.6
Ether extract	92.0	91.0	90.3	91.9	95.1	84.9	85.9	92.6	94.9	92.5
Total fiber	29.5	29.1	20.1	20.4	21.3	18.0	20.2	22.9	15.1	10.6
Ash	111.2	109.0	109.7	108.7	112.4	97.5	102.2	91.8	96.5	95.6
Nitrogen-free extract[t]	312.4	311.1	321.6	325.5	329.4	362.6	345.3	347.7	338.8	326.3
Gross energy (MJ kg^{-1})[u]	19.52	19.52	19.51	19.59	19.60	19.54	19.49	19.82	19.86	19.84

Table 3 (continued)

[a]0-R, reference diet with 150.0 g kg^{-1} fish meal; 25-R, diet with 25% replacement of fish meal for *Scenedesmus* sp.; 50-R, diet with 50% replacement of fish meal for *Scenedesmus* sp.; 75-R, diet with 75% replacement of fish meal for *Scenedesmus* sp.; 100-R, diet with 100% replacement of fish meal for *Scenedesmus* sp.; 0-I, control diet containing 120.0 g kg^{-1} fish meal; 150-I, plant-based diet with 150 g kg^{-1} *Scenedesmus* sp.; 200-I, plant-based diet with 200 g kg^{-1} *Scenedesmus* sp.; 250-I, plant-based diet with 250 g kg^{-1} *Scenedesmus* sp.; 300-I, plant-based diet with 300 g kg^{-1} *Scenedesmus* sp.

[b]Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil). 105.2 g kg^{-1} moisture, 478.8 g kg^{-1} crude protein (CP), 20.4 g kg^{-1} ether extract (EE), 5.6 g kg^{-1} fiber, 65.8 g kg^{-1} ash, 6.2 g kg^{-1} methionine (Met), 29.1 g kg^{-1} lysine (Lys), 13.0 g kg^{-1} methionine + cysteine (M + C)

[c]J. Macedo (Fortaleza, Brazil). 93.7 g kg^{-1} moisture, 108.2 g kg^{-1} CP, 11.0 g kg^{-1} EE, 4.4 g kg^{-1} fiber, 5.0 g kg^{-1} ash, 1.8 g kg^{-1} Met, 2.3 g kg^{-1} Lys, 4.1 g kg^{-1} M + C

[d]Pesquera Pacific-Star (Puerto Montt, Chile). See Table 2 for composition

[e]Algae Biotecnologia Ltda. (Holambra, Brazil). See Table 2 for composition

[f]Sementes Selecta S.A. (Araguari, Brazil). 72.3 g kg^{-1} moisture, 616.4 g kg^{-1} CP, 23.5 g kg^{-1} EE, 33.1 g kg^{-1} fiber, 68.0 g kg^{-1} ash, 8.0 g kg^{-1} Met, 37.6 g kg^{-1} Lys, 16.2 g kg^{-1} M + C

[g]Amytex 100. Tereos Syral S.A.S. (Marckolsheim, France). 67.5 g kg^{-1} moisture, 787.1 g kg^{-1} CP, 16.9 g kg^{-1} EE, 5.6 g kg^{-1} fiber, 7.9 g kg^{-1} ash, 12.2 g kg^{-1} Met, 13.7 g kg^{-1} Lys, 28.0 g kg^{-1} M + C

[h]ADM do Brasil Ltda. (Campo Grande, Brazil)

[i]Nymega™ DHA-rich algal biomass, Helia® (Gilbert, USA). Composed of 99.8% of *Aurantiochytrium* spp. Guarantee levels according to manufacturer: > 50 g kg^{-1} moisture, > 50 g kg^{-1} CP, > 650 g kg^{-1} EE, < 120 g kg^{-1} ash, > 3.0 g kg^{-1} phosphorous, < 2.5 g kg^{-1} EPA (eicosapentaenoic acid, 20:5n-3), < 60 g kg^{-1} DPA (docosapentaenoic acid, 22:5n-6), > 200 g kg^{-1} DHA (docosahexaenoic acid, 22:5n-3). Sample supplied by Syndel International Inc. (Ferndale, WA, USA)

[j]Qrill™ AQUA, Antarctic krill meal (full fat), Aker Biomarine Feed Ingredients AS (Lysaker, Norway). 66.1 g kg^{-1} moisture, 570.5 g kg^{-1} CP, 184.7 g kg^{-1} EE, 118.2 g kg^{-1} ash, 16.3 g kg^{-1} Met, 39.2 g kg^{-1} Lys, 20.8 g kg^{-1} M + C

[k]97.5 g kg^{-1} moisture, 831.3 g kg^{-1} CP, 56.5 g kg^{-1} EE, 23.4 g kg^{-1} Met, 50.6 g kg^{-1} Lys, 31.7 g kg^{-1} M + C

[l]Vaccinar Industria e Comercio Ltda. (Pinhais, Brazil). Guarantee levels per kg of product: vitamin A, 1,200,000 IU; vit. D3, 200,000 IU; vit. E, 60,000 mg; vit. K3, 1,000 mg; vit. B1, 2,400 mg; vit. B2, 2,400 mg; vit. B6, 6,000 mg; vit. B12, 4 mg; nicotinic acid, 10,000 mg; pantothenic acid, 5,200 mg; biotin, 20 mg; folic acid, 400 mg; vit. C, 30,000 mg; choline, 50,000 mg; inositol, 80,000 mg; Fe 26,000 mg; Cu, 2,000 mg; Zn, 20,000 mg; Mn, 5,000 mg; Se, 100 mg; I, 600 mg; Co, 105 mg; Cr, 60 mg

[m]Nutri-Bind Aqua Veg Dry, Nutri-Ad International NV (Dendermonde, Belgium). Synthetic binder consisting of calcium lignosulfonate (940 g kg^{-1}) and guar gum (60 g kg^{-1})

[n]MetAMINO®, Evonik Nutrition and Care GmbH (Hanau, Germany). DL-Methionine 990 g kg^{-1}

[o]Biolys®, Evonik Nutrition and Care GmbH (Hanau, Germany). L-Lysine 546 g kg^{-1}

[p]ThreAMINO®, Evonik Nutrition and Care GmbH (Hanau, Germany). L-Threonine, 985 g kg^{-1}

[q]Choline chloride 600 g kg^{-1}, Indukern do Brasil Química Ltda. (Jundiaí, Brazil)

[r]Cholesterol SF, Dishman Netherlands B.V. (Veenendaal, Netherlands). Minimum of 910 g kg^{-1} of active cholesterol

[s]Rovimix® Stay C® 35. Minimum of 350 g kg^{-1} of phosphorylated vitamin C activity. DSM Nutritional Products AG (Schweiz, Switzerland)

[t]Nitrogen-free extract. Calculated by difference [dry matter – (CP + EE + CF + ash)]

[u]Calculated as GE = (4,143 + (560 × EE) + (150 × CP) – (440 × ash)) × 0.0041868

EPA (eicosapentaenoic acid, 20:5n-3), and ARA (arachidonic acid, 20:4n-6), but contained higher levels of DHA (between 5.0 and 5.5 g kg^{-1} of the diet, as-is basis) than the control (0-I) diet (1.6 g kg^{-1}). The dietary levels of LOA (linoleic acid, 18:2n-6) and LNA

Table 4 Amino acid composition of experimental diets. CV, coefficient of variation

Amino Acids	Diets (g kg^{-1} of the diet, as-is)/Study Phase I						Diets (g kg^{-1} of the diet, as-is)/Study Phase II					
	0-R	25-R	50-R	75-R	100-R	CV (%)	0-I	150-I	200-I	250-I	300-I	CV (%)
Essential amino acids (EAA)												
Arginine	23.0	22.8	21.1	21.5	21.3	4.1	20.3	20.3	19.6	19.9	19.8	1.6
Histidine	9.0	8.6	7.7	7.9	7.6	7.5	7.7	7.2	6.8	6.6	6.5	7.1
Isoleucine	15.7	15.7	14.6	15.0	15.3	3.1	14.2	14.4	13.7	13.6	14.0	2.4
Leucine	27.5	27.4	25.9	27.1	27.4	2.5	25.1	26.2	25.6	26.5	27.3	3.2
Lysine	20.4	19.7	19.7	20.4	20.3	1.8	20.2	21.6	20.7	22.4	24.1	7.1
Methionine	9.5	8.9	8.6	9.0	8.6	4.1	7.9	8.1	8.0	8.6	8.9	5.2
Met + Cysteine	16.5	14.8	13.0	13.3	11.6	13.5	12.6	12.7	12.5	13.1	13.4	2.9
Phenylalanine	17.8	17.8	17.1	18.0	18.5	2.8	16.7	17.9	17.2	17.4	18.0	3.1
Threonine	13.7	13.8	12.9	13.5	13.2	2.8	13.5	14.0	14.1	15.0	15.8	6.3
Tyrosine	11.7	11.7	12.5	12.4	12.5	3.5	11.7	11.7	11.8	11.9	12.5	2.8
Valine	17.7	17.8	15.8	16.0	16.6	5.6	15.0	15.8	15.7	15.9	16.9	4.3
Non-essential amino acids (NEAA)												
Alanine	18.0	18.2	18.0	18.7	19.0	2.4	16.2	17.4	17.8	19.6	20.7	9.8
Aspartic acid	7.0	5.9	4.4	4.3	3.0	31.5	4.7	4.6	4.5	4.5	4.5	2.0
Cystine	21.4	20.3	18.2	17.8	16.9	9.9	18.3	15.9	16.0	17.2	17.6	6.1
Glycine	17.2	16.7	15.9	16.7	15.8	3.6	16.2	15.0	14.4	14.8	14.3	5.1
Glutamic acid	22.8	22.0	20.9	20.8	20.4	4.6	22.5	21.6	21.7	21.6	23.1	3.1
Proline	30.6	33.8	31.6	32.0	31.4	3.7	30.5	28.8	28.6	27.9	27.4	4.1
Serine	69.2	69.0	61.8	63.0	61.1	6.1	67.1	62.2	61.0	60.4	59.8	4.7
Taurine	1.6	1.3	0.8	0.5	0.2	65.0	1.1	-	-	-	0.2	183.7
Sum EAA	166.0	164.2	155.9	160.8	161.3	2.4	152.3	157.2	153.2	157.8	163.8	2.9
Sum NEAA	187.8	187.2	171.6	173.8	167.8	5.2	176.6	165.5	164.0	166.0	167.6	3.0
EAA + NEAA	353.8	351.4	327.5	334.6	329.1	3.7	328.9	322.7	317.2	323.8	331.4	1.7

Table 5 Fatty acid profile of diets used in study phase II

Fatty acid	Diets/Fatty acid content (g kg^{-1} of the diet, as-is)				
	0-I	150-I	200-I	250-I	300-I
14:0	0.6	0.6	0.7	0.6	0.8
15:0	0.1	0.1	0.1	0.1	0.1
16:0	12.4	17.1	18.6	19.3	19.5
17:0	0.2	0.3	0.3	0.3	0.2
18:0	3.1	2.6	2.7	2.6	2.6
20:0	0.2	0.2	0.2	0.2	0.3
21:0	0.3	0.1	0.1	0.1	0.1
22:0	0.3	0.3	0.3	0.3	0.3
23:0	-	0.1	0.1	0.1	0.1
24:0	0.2	0.2	0.2	0.2	0.2
16:1n-7	-	0.2	0.2	0.3	-
18:1n-9	18.5	11.4	11.8	11.7	11.2
20:1n-9	0.6	0.1	0.1	0.1	0.1
22:1n-9	0.1	-	-	-	-
24:1n-9	0.1	-	-	-	-
18:2n-6	39.5	39.6	41.9	41.7	40.3
18:3n-6	0.1	-	-	-	-
18:3n-3	4.7	7.8	9.6	11.6	11.1
20:3n-6	0.2	-	-	-	-
20:3n-3	0.1	-	-	-	-
20:4n-6	0.2	-	-	-	-
20:5n-3	1.0	-	-	-	-
22:6n-3	1.6	5.1	5.5	5.5	5.0
n-3[a]	7.4	12.9	15.1	17.1	16.1
n-6[b]	40.0	39.6	42.0	41.7	40.3
n-9[c]	19.3	11.5	11.9	11.8	11.3
SFA[d]	17.4	21.6	23.3	23.8	24.2
MUFA[e]	20.1	11.7	12.1	12.1	11.3
PUFA[f]	44.6	47.4	51.5	53.3	51.4
HUFA[g]	2.8	5.1	5.6	5.5	5.0
EFA[h]	7.3	12.9	15.1	17.1	16.1

[a]n-3, 18:3n-3, 20:3n-3, 20:5n-3, 22:6n-3

[b]n-6, 18:2n-6*c*, 18:3n-6, 20:3n-6, 20:4n-6, 22:2n-6

[c]n-9, 18:1n-9, 20:1n-9, 22:1n-9, 24:1n-9

[d]SFA, saturated fatty acids, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0

[e]MUFA, monosaturated fatty acids, 16:1, 18:1, 20:1, 22:1, 24:1

[f]HUFA, highly unsaturated fatty acids, 20:4, 20:5, 22:6

[g]EFA, essential fatty acids, 18:2n-6, 18:3n-3, 20:5n-3, 22:6n-3

(linolenic acid, 18:3n-3) fell between 43.57 (300-I) and 46.53 g kg^{-1} (0-I) and 5.54 (0-I) and 12.0 g kg^{-1} (300-I), respectively.

Rearing system and water preparation

The rearing systems consisted of circular tanks, made of blue polypropylene, equipped with an independent water inlet and outlet. For study phases I and II, 0.5-m^3 indoor (0.57 m^2 bottom area, 0.56 m height) and 1-m^3 outdoor (bottom area of 1.02 m^2; 0.83 m height) tanks were used, respectively. Tanks had perforated lids to prevent shrimp escape and limit sunlight exposure. The indoor system maintained a 12-h artificial light cycle starting at 05:45 am. Tanks were equipped with continuous aeration delivered to the tank bottom through an air diffusion system. Both rearing systems operated under constant water recirculation with settling reservoirs to allow partial removal of organic matter to control accumulation of nitrogen compounds. No mechanical filtration was carried out. Rearing tanks in each system were connected to a 10-m^3 settling reservoir and a 20-m^3 reservoir for water storage and redistribution with a mechanical pump.

Sand-filtered seawater from a nearby estuary was used for tank filling. Two weeks before shrimp stocking, the water was fertilized with dried sugar-cane molasses and ground shrimp feed to stimulate growth of nitrifying bacteria. Fertilization consisted of applying 4.5 g m^{-3} of dried sugar-cane molasses together with 10 g m^{-3} of ground shrimp feed over a continuous five-day period. After fertilization, water was strongly aerated for a week, when a brownish color was observed.

During the rearing period, dried sugar-cane molasses was applied at 4.5 g m^{-3} once a week. Water pH, temperature, and salinity were monitored once a day starting at 09:00 am in all tanks. In study phase I, salinity, temperature, and pH reached 34 ± 1.6 g L^{-1} (30 – 37 g L^{-1}, $n = 2{,}404$), 28.8 ± 0.5 °C (27.4 – 29.9 °C, $n = 2{,}364$), and 8.1 ± 0.4 (7.4 – 8.8, $n = 2{,}404$), respectively. In phase II, these parameters reached 43 ± 2.3 g L^{-1} (37 – 47 g L^{-1}, $n = 1{,}820$), 29.7 ± 0.8 °C (26.8 – 31.9 °C, $n = 1{,}785$) and 8.5 ± 0.1 (8.0 – 8.8, $n = 1{,}820$), respectively.

Shrimp stocking and feeding

Post-larvae (PL) of *P. vannamei* arrived in the lab as PL10 (10-day old PL) and were nursery-reared until juvenile. In study phase I, a total of 2,000 shrimp of 1.99 ± 0.15 g (coefficient of variation, cv = 7.6%) were stocked under 88 animals m^{-2} (50 shrimp $tank^{-1}$) in 40 indoor tanks. For study phase II, a total of 3,570 shrimp of 1.78 ± 0.16 g (cv = 9.1%) were stocked under 100 animals m^{-2} (102 shrimp $tank^{-1}$) in thirty-five 1-m^3 outdoor tanks.

In the indoor tanks, shrimp were manually fed using circular feeding trays measuring 141 mm in surface area with borders 35 mm in height. Each tank had one tray placed in the middle. At each feeding, trays were inspected to detect the presence of uneaten food from the previous meal, which, when observed, was collected for weighing and disposal. Shrimp received four daily feedings at 07:00 am, 10:00 am, 01:00 pm, and 04:00 pm, with meal proportions of 25%, 15%, 15%, and 45% at the 1st, 2nd, 3rd, and 4th feeding times, respectively. In outdoor tanks, shrimp were fed 10 times a day with an automatic feeder programmed to deliver feed between 07:00 am and 05:00 pm (Nunes et al. 2019).

The daily feed rations were determined using the equation $MM = 0.0931BW^{0.6200}$, where MM is the maximum amount of feed that can be consumed daily by an individual with a specific BW (Nunes and Parsons 2000). To control feed conversion ratio (FCR), daily meals were reduced by 30% across all treatments (Nunes et al. 2006). Initially, meals were adjusted following an individual BW gain of 100 mg day^{-1} and a fixed

weekly reduction in shrimp survival of 0.30 and 0.38% (phases I and II, respectively). Starting on the 14th day of rearing and then on a weekly basis, a total of 10 shrimp per tank was captured, weighed individually, and returned to their respective tank. Until the next sampling, meals were adjusted assuming an average daily weight gain achieved in the previous week for each specific rearing tank, maintaining a fixed weekly drop in shrimp survival across all diets. Dead animals were not replaced throughout the culture period.

Growth performance

At harvest, all live shrimp were counted and individually weighed to a 0.01-g precision. Final shrimp survival (S, %) was calculated as: (POPf/POPi) × 100, where: POPi = number of stocked shrimp and POPf = number of shrimp at harvest. The weekly weight gain (WWG, g week^{-1}) was determined by the formula: WWG = [(BWf – Bwi)/t] × 7, where BWi = wet shrimp body weight (BW, g) at stocking, BWf = final shrimp BW at harvest, and t = number of days in culture. The gain in shrimp yield (YIE, g of shrimp biomass gained m^{-2}) was determined as: (BIOf – BIOi)/tank bottom area (m^2), where BIOi = initial shrimp biomass per tank (g), BIOf = final shrimp biomass (g), and tank bottom area. FCR was calculated by dividing the total inputs of feed (g, DM basis) delivered per tank during the entire rearing period by the total gained shrimp biomass per tank (g, as-is). The apparent feed intake (AFI, g of feed delivered divided by the number of stocked shrimp) was calculated by dividing the total amount of feed delivered (g, DM) by the number of stocked shrimp.

Feed preference

Feed preference was assessed following the methodology described by Browdy et al. (2012) and Nunes et al. (2019). The method consisted of simultaneously confronting all diets and measuring their relative apparent feed intake (RAFI, %). In this study, five tanks of 0.5 m^3 were stocked with juvenile shrimp of 13.38 ± 1.95 g (n = 250) under 88 animals m^{-2} (50 shrimp tank^{-1}). Five feeding trays (141 mm^2 in surface area) were placed in each tank with individual diets prepared for study phase I. Feeding trays were simultaneously immersed in water rested in the tank bottom near the side walls of the tank and away from the aeration area. During the observation period, shrimp were fed in excess, so that feed remains were always available for collection. Feed delivery and collection of uneaten feed took place at the following times: 1st meal: 07:30 am–08:30 am; 2nd meal: 01:30 pm–02:30 pm, respectively.

During shrimp feeding, rearing tanks operated with continuous water exchange and filtration. This allowed water to remain clear during the complete observation period which lasted seven continuous days. After one hour of feed delivery, feeding trays were recovered, feed remains collected, dried in a convection oven at 105°C for 24 h and weighed. RAFI (% of the total meal consumed) was calculated as: (total amount of dried feed remains collected, in g/total amount of dried feed delivered, in g) × 100. The total amount of feed consumed was calculated on a DM basis by subtracting the amount of dried feed leftovers from the dried amount of feed delivered. Feed moisture content was determined by drying five samples of 3 g of each diet type in a convection oven at 105°C for 24 h.

Statistical analyses

Statistical analyzes were performed with the Statistical Package for Social Sciences, package 23 (IBM® SPSS® Statistics, Chicago, Illinois, USA). The effect of the dietary treatment on shrimp growth performance and feed preference were analyzed using One-way ANOVA. When significant differences were detected, pairwise comparisons were performed using the Tukey´s HSD test. The significant level of 5% was set in all statistical analyses.

Results

In study phase I, final shrimp survival, weekly growth rate, gained yield, and FCR reached a mean of 96.6 ± 3.4%, 1.09 ± 0.05 g, 982 ± 47 g m^{-2}, and 1.56 ± 0.07, respectively (Table 6). No significant effect on these parameters was observed following the partial or total replacement of FM with SCM ($P > 0.05$). However, a statistically higher AFI was found when shrimp were fed diet 100-R, except when compared to 75-R. Shrimp were harvested with a mean final BW of 13.66 ± 0.54 g (Fig. 1). No significant differences were found in final BW between dietary treatments.

In study phase II, final shrimp survival (94.3 ± 3.8%) was also unaffected by diet type ($P > 0.05$; Table 6). Also, no statistically significant differences in weekly growth rate (0.85 ± 0.02 g) were detected between shrimp fed fish-free diets and the reference diet (0-I) with 120 g kg^{-1} FM ($P > 0.05$). However, shrimp BW at harvest varied statistically. The highest final BW was observed in shrimp fed the 0-I diet (12.24 ± 0.93 g) compared to remaining diets (Fig. 1). Shrimp fed diet 200-I, *i.e.*, with a dietary inclusion of 200 g kg^{-1} SCM, reached a final BW (10.51 ± 0.39 g) higher than those fed diet 300-I (9.25 ± 1.00 g). The final BW of shrimp fed the remaining fish-free diets showed no significant variation. The highest gained yield (921 ± 70 and 828 ± 45 g m^{-2}), and the lowest FCR (1.52 ± 0.10 and 1.61 ± 0.08) was found for shrimp raised with the 0-I and 200-I diets, respectively. AFI on the other hand, dropped significantly in all fish-free diets compared to the 0-I.

The feed preference assay clearly showed a higher RAFI for the reference diet (0-R, 74.6 ± 2.2%, mean ± standard error) compared to all other diets, regardless of the FM replacement level ($P < 0.05$; Fig. 2). AFI remained relatively uniform among diets 25-R (52.2 ± 2.1%), 50-R (43.8 ± 2.1%), and 75-R (46.0 ± 2.5%). However, there was a further drop in AFI to 42.0 ± 2.6% when FM was completely removed from the diets, *i.e.*, diet 100-R with a dietary inclusion of 150 g kg^{-1} SCE and no FM. However, AFI for this diet did not differ from diet 50-R.

Discussion

The present study demonstrated that the protein from SCM can completely replace FM protein (on a weight-to-weight basis, w/w) in diets for juvenile *P. vannamei*. FM replacement with SCM was achieved by formulating diets with CAA supplementation, meeting shrimp requirements for n-3 HUFAs and incorporating feed attractants. Under our experimental conditions, diets containing between 37.5 and 150 g kg^{-1} SCM

Table 6 Performance of juvenile *P. vannamei* after 75 and 71 days of rearing fed diets containing *Scenedesmus* sp. meal in partial or total replacement of fish meal (study phase I) or in fish-free diets (study phase II), respectively. Data presented as mean ± standard deviation obtained from a total of 40 indoor and 35 outdoor tanks, respectively. Common letters indicate non-statistically significant differences according to Tukey's HSD at $\alpha = 0.05$

	Diets/Study Phase I					*P* ANOVA
Performance	0-R[a]	25-R	50-R	75-R	100-R	
Initial body weight (g)	1.99 ± 0.16	2.00 ± 0.15	1.98 ± 0.15	2.01 ± 0.15	1.99 ± 0.15	0.149
Final survival (%)	97.0 ± 3.16	96.3 ± 3.53	96.3 ± 2.73	94.8 ± 3.99	98.8 ± 1.39	0.210
Growth (g week^{-1})	1.08 ± 0.03	1.08 ± 0.05	1.09 ± 0.05	1.11 ± 0.08	1.09 ± 0.03	0.700
Gained yield (g m^{-2})	982 ± 46	965 ± 48	977 ± 42	978 ± 58	1,009 ± 41	0.447
AFI (g of feed shrimp^{-1})[b]	17.4 ± 0.20[a]	17.3 ± 0.15[a]	17.3 ± 0.22[a]	17.5 ± 0.20[ab]	17.7 ± 0.14[b]	< 0.0001
FCR[c]	1.55 ± 0.06	1.57 ± 0.07	1.55 ± 0.06	1.57 ± 0.08	1.54 ± 0.06	0.922
	Diets/Study Phase II					*P* ANOVA
Performance	0-I[a]	150-I	200-I	250-I	300-I	
Initial body weight (g)	1.78 ± 0.16	1.77 ± 0.16	1.78 ± 0.17	1.78 ± 0.16	1.78 ± 0.16	0.426
Final survival (%)	92.3 ± 6.0	95.1 ± 3.8	95.7 ± 3.7	94.3 ± 2.4	93.7 ± 2.9	0.582
Growth (g week^{-1})	0.85 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	0.84 ± 0.03	0.85 ± 0.01	0.508
Gained yield (g m^{-2})	921 ± 70[c]	756 ± 45[ab]	828 ± 45[bc]	745 ± 47[ab]	688 ± 91[a]	< 0.0001
AFI (g of feed shrimp^{-1})[b]	13.92 ± 0.37[c]	12.75 ± 0.22[ab]	13.27 ± 0.27[b]	12.89 ± 0.34[ab]	12.51 ± 0.51[a]	< 0.0001
FCR[c]	1.52 ± 0.10[a]	1.69 ± 0.07[bc]	1.61 ± 0.08[ab]	1.73 ± 0.06[bc]	1.84 ± 0.18[c]	< 0.0001

[a]Diets 0-R and 0-I contained 150 and 120 g kg^{-1} fish meal, respectively

[b]Apparent feed intake given as g of feed consumed per number of stocked shrimp

[c]Feed conversion ratio

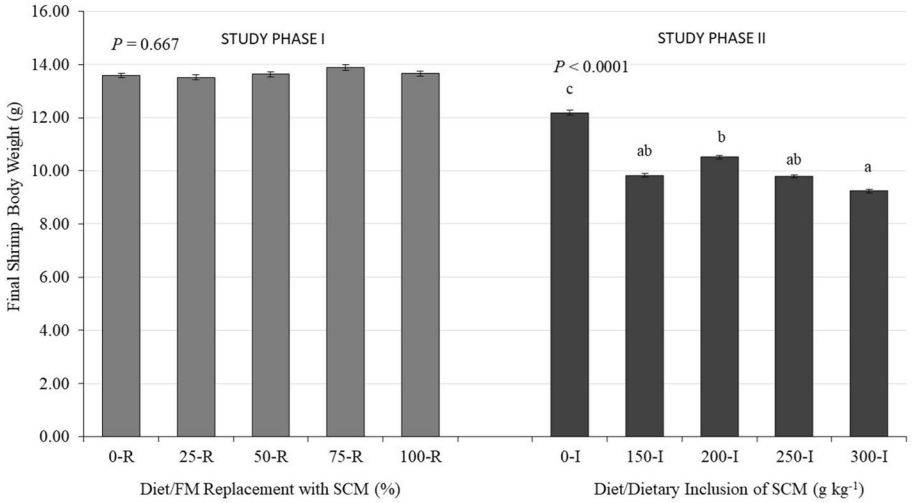

Fig. 1 Mean final shrimp body weight (g, ± standard error) of juvenile *P. vannamei* after 75 and 71 days of rearing in study phases I and II, respectively. In phase I, fish meal (FM) was progressively replaced by *Scenedesmus sp.* (SCM) from 0% (0-R) to 100% (100-R). In phase II, the dietary inclusion of SCM increased from 150 to 300 g kg^{-1}. Diets 0-R and 0-I contained 150 and 120 g $kg.^{-1}$ FM, respectively. Common letters indicate non-statistically significant differences according to Tukey's HSD at $\alpha = 0.05$

resulted in shrimp exhibiting final survival, growth rate, final BW, and feed efficiency equivalent to those fed a diet with 150 g kg^{-1} FM.

Previous studies have already shown the suitability of SCM as a dietary protein source for gilthead sea bream (*Sparus aurata*, Vizcaíno et al. 2014), rainbow trout (*Oncorhynchus mykiss*, Tomás-Almenar et al. 2018, Skalli et al. 2020), and Atlantic salmon (*Salmo salar*, Gong et al. 2019). Other sources of microalgae meal, derived from *Haematococcus pluvialis* (Ju et al. 2012), *Spirulina platensis* (Macias-Sancho et al. 2014; Pakravan et al. 2017), and *Aurantiochytrium sp.* (Guimarães et al. 2019), have all been considered viable feed ingredients to partially replace FM or fish oil in diets for juvenile *P. vannamei*. However, this is the first time the effectiveness of SCM has been reported as a primary dietary protein source for juvenile whiteleg shrimp.

Nevertheless, Phase II of this study revealed significant limitations when marine proteins and oils were entirely replaced with plant-derived ingredients and microalgae meals (SCM and AUM), and marine oils with soybean oil. This major change in diet formulation resulted in poorer shrimp performance, as evidenced by decreased yield, feed intake, final body weight, and a higher FCR. The reference diet (0-I), which contained a balance of SBM, FM, SPC, and fish oil, yielded better results due to its well-balanced nutritional profile, including both n-3 HUFAs and palatability- and attractability-enhancing compounds present in marine ingredients.

The observed decline in performance in diets lacking FM and fish oil was initially attributed to a deficiency in EPA. This was particularly concerning as the DHA-rich algal extract, composed of 99.8% *Aurantiochytrium spp*., contained no EPA and was the only source of n-3 HUFAs in those diets. At 15 g kg^{-1}, this ingredient led to a dietary DHA concentration of 5.3 g kg^{-1} on an as-is basis, with no detectable EPA. While the absence of EPA could be linked to the reduced performance, the ability of some aquatic species to interconvert fatty acids suggests that the roles of EFAs, such as EPA and DHA, in this

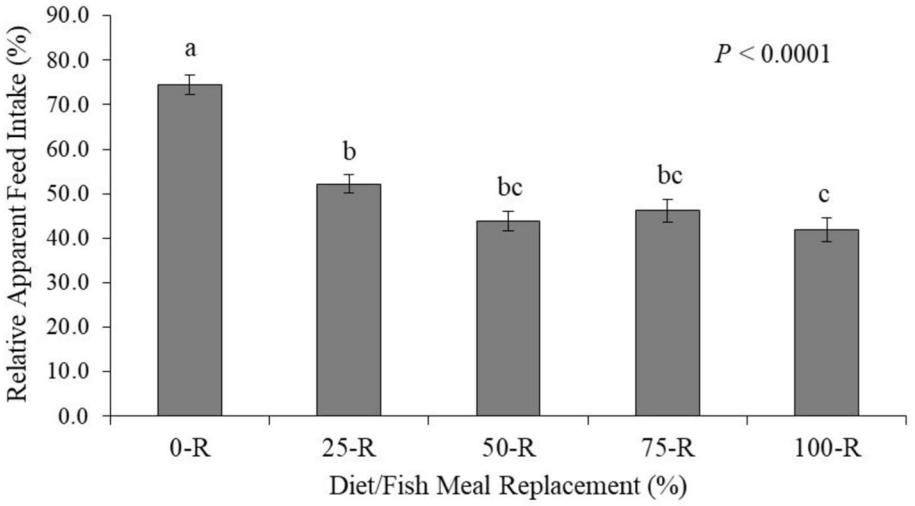

Fig. 2 Relative apparent mean feed intake (±standard error) of five diets with progressive replacement levels of fish meal (FM) with *Scenedesmus* sp. meal (SCM). Different letters in each column indicate statistically significant differences according to Tukey's HSD at $\alpha = 0.05$

context require further investigation (Bou et al. 2017; Stubhaug et al. 2005; Glencross & Smith 2001; Ruyter and Torstensen 1999).

The dietary requirements for EPA and DHA in penaeid shrimp, whether added individually or together, are well-documented (Glencross and Smith 2001; Kanazawa et al. 1985; Gonzalez-Felix et al. 2002). However, these requirements for EFAs vary significantly among different species of penaeid shrimp, influencing their growth and development differently. For instance, *Penaeus japonicus*, *Penaeus monodon*, and *P. vannamei* exhibit distinct variations in their EFA requirements, both qualitatively and quantitatively.

In *P. japonicus*, optimal growth is achieved with specific inclusion levels of EPA and DHA, highlighting their importance as EFAs (Kanazawa et al. 1978; Kanazawa 1984). In contrast, *P. monodon* demonstrates a complex interaction between different fatty acids, with DHA identified as the most important EFA (Merican and Shim 1996). Research indicates that growth improves with the separated inclusion of both EPA and DHA, and the overall requirement is lower when both are present together in the diet (Glencross and Smith 2001). *P. monodon* requires both n-3 and n-6 fatty acids, with optimal growth achieved at specific ratios and combinations, indicating a more intricate requirement profile compared to *P. japonicus* (Glencross and Smith 1999; Glencross et al. 2002).

For *P. vannamei*, previous studies reported that inclusion of DHA or a mix of n-3 HUFAs significantly enhanced growth, demonstrating a requirement for these longer-chain fatty acids over shorter-chain ones like LOA and LNA (Gonzalez-Felix et al. 2002, 2003). Thus, the nutritional requirements of *P. vannamei* are distinct, with a lower overall requirement for dietary EFA compared to *P. japonicus* and *P. monodon*. Although studies in *P. vannamei* have not adopted a nutrient-deletion approach in purified diets to rank the EFA by order of importance, there seems to be a common understanding that, for this species, if the total n-3 HUFA requirements are met, no negative impact on growth should be expected. González-Félix et al. (2002) identified a dietary requirement of approximately

5 g kg^{-1} or less of total n-3 HUFA for juvenile *P. vannamei*, aligning with the dietary levels of n-3 HUFAs found in our fish-free diets (150-I to 300-I).

Adequate growth performance of juvenile *P. vannamei* fed fish oil-free diets has been recently reported by Araújo et al. (2019). However, their diets still contained an n-3 HUFA source (100 g kg^{-1} FM) combined with an algae-derived DHA concentrate meal, resulting in some dietary EPA content. Another study by Wang et al. (2015) demonstrated that a DHA-rich microalgae biomass (*Schizochytrium sp.*) could effectively replace marine fish oil in *P. vannamei* diets when FM was maintained at 350 g kg^{-1}, still with dietary EPA present. In contrast, our fish-free diets, apart from the DHA-rich algal extract, completely lacked any other n-3 HUFA sources.

The fatty acid composition of our formulations is more closely aligned with those presented by Guimarães et al. (2019). Their study progressively substituted cod oil with a DHA algal extract derived from *Aurantiochytrium spp*. in diets for juvenile *P. vannamei*. Although their algal DHA source differs from the one used in our study, both belong to the same genus and share similarities in their fatty acid composition. The algal extract used by Guimarães et al. (2019) also lacked EPA and contained 272 g kg^{-1} DHA (on a DM basis). Their research showed that *Aurantiochytrium spp.* meal could totally replace cod oil. Notably, a diet with 100% replacement of cod oil, without dietary EPA, did not compromise shrimp growth performance, yielding results equivalent to the control diet with 40.0 g kg^{-1} cod oil. This suggests that the poorer performance in Phase II is most likely due to factors other than an EPA or n-3 HUFA dietary deficiency.

Marine ingredients, such as fish meal, krill meal, squid meal, and fish oil, contain compounds like free amino acids, nucleotides, and specific peptides that are highly palatable and stimulate feeding in shrimp (Suresh et al. 2011). Previous studies have reported that inadequate replacement of these attractants leads to lower feed consumption, poor growth rates, and overall diminished productivity in shrimp. In Phase II of this study, this effect was evident by the reduced AFI in fish-free diets, with the reference diet (containing 120 g kg^{-1} FM and 12 g kg^{-1} fish oil) showing the highest RAFI ($P < 0.05$). Compared to diets tested in Phase I, the fish-free diets posed additional challenges to feed attractability, as both squid meal and krill meal were also removed, and dietary levels of 150 g kg^{-1} or more of SCM were adopted.

Supporting these observations, Sarker et al. (2020) reported a drop in feed intake when evaluating the digestibility of *Nannochloropsis sp.* and *Isochrysis sp.* in fish-free diets for rainbow trout. Conversely, Gong et al. (2019) found no negative effect on feed intake in Atlantic salmon when incorporating up to 200 g kg^{-1} SCM in low FM diets (25 to 50 g kg^{-1}), indicating that the negative effect of SCM on feed intake may be related to inclusion levels and the complete absence of potential feed attractants. Further emphasizing the importance of feed attractability, Silva-Neto et al. (2012) found that *Spirulina sp.* meal included at only 50 g kg^{-1} in a diet for juvenile *P. vannamei* with 139 g kg^{-1} FM performed as effectively as a commercial feed attractant composed of a complex amino acid mixture with enzymatically digested bivalve mollusk. These results suggest that feed devoid of attractants and associated with high microalgae inclusion levels might contribute to the observed decline in shrimp performance. More studies are needed to comprehensively understand the effects of high inclusions of SCM in combination with AUM in shrimp diets, particularly regarding their impact on feed attractability.

Conclusions

This study has demonstrated that *Scenedesmus sp.* (SCM) can effectively replace fish meal (w/w) in diets for juvenile *Penaeus vannamei* when balanced for essential amino acids and fatty acids. Diets containing 37.5 to 150 g kg^{-1} SCM achieved comparable growth performance to those with 150 g kg^{-1} FM. However, the complete replacement of marine proteins and oils with plant-derived ingredients and microalgae-based meals in Phase II revealed significant limitations, including reduced feed intake, feed efficiency, and overall shrimp yield. The absence of palatability- and attractability-enhancing compounds, which are abundant in marine-origin ingredients, likely contributed as the primary cause of these negative effects.

The findings underscore the importance of maintaining a balanced nutritional profile that includes elements to enhance feed attractability for optimal shrimp performance. Future research should focus on refining fish-free diet formulations containing SCM and AUM to ensure they meet all essential nutrient requirements and improve feed attractability functionality. Addressing these challenges will be crucial for advancing sustainable and economically viable shrimp aquaculture practices.

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A.N. was responsible for the conceptualization, data curation, funding acquisition, and project administration. A.N. also managed resources and supervised the project. Both A.N., F.F., and J.L. engaged in data curation to ensure accuracy. During the investigation phase, A.N., J.L., and A.D. collaborated for a comprehensive exploration. Project administration responsibilities were shared by A.N. and J.L., with resource management jointly handled by A.N. and J.L.. F.F., and J.L. collaborated on the original manuscript draft. In the final stages, A.N., F.F., and A.D. contributed to the manuscript review and editing, enhancing clarity before submission.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethics Statement Ethical review and approval were waived for this study, since shrimp do not fall under phylum Chordata and subphylum Vertebrata which require ethical approval for research activities according to the Brazilian Federal Law No. 11794 of 8 October 2008 (http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2008/lei/l11794.htm) and Brazilian Federal Decree No. 6899 of 15 July 2009 (http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2009/decreto/d6899.htm). All rearing procedures were performed in compliance with relevant local laws and institutional guidelines, including those related to animal welfare. All applicable international, national, and institutional guidelines for the care and use of animals were followed by the authors.

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