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Research Article

Aurantiochytrium sp. and *Nannochloropsis* spp. meals as substitutes for fish oil in practical diets for Pacific white shrimp *Penaeus vannamei*

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ABSTRACT. This study aimed to evaluate the use of microalgae meal (*Aurantiochytrium* sp. and *Nannochloropsis* spp.) as a substitute for fish oil in the diet of shrimp *Penaeus vannamei* reared in a clear water system. In the diet of these animals, the concentration of fish oil decreased as microalgae meal was inserted, and these were used in five different substitutions: 0, 25, 50, 75, and 100%. The treatments were carried out in triplicate, and after 49 days of cultivation, the zootechnical parameters and the composition of fatty acids in the animals' muscles were analyzed. In zootechnical performance, the shrimp obtained an average final weight of 16.59 ± 0.3 g, and the growth was approximately 1.89 ± 0.30 g week^{-1}. In addition, feed conversion was, on average, 1.47 ± 0.28, and survival was greater than 97%. However, no significant difference was observed in these parameters. Regardless of the levels of fish oil replacement by microalgae meal, the composition of fatty acids in the shrimp muscle was ensured, as well as their quantity within groups, such as n-3 and n-6 polyunsaturated fatty acids. Thus, with the results obtained in the present study, it is suggested that it is possible to formulate a high-performance diet without any ingredients of marine origin (fish oil or fish meal), contributing to aquaculture's sustainability.

Keywords: *Penaeus vannamei*; *Aurantiochytrium* sp.; *Nannochloropsis* spp.; DHA; EPA; algae meal; LC-PUFA

INTRODUCTION

Fish oil and meal are among the main ingredients used in shrimp feed, and compared to other activities, aquaculture (especially shrimp farming) has the highest demand for these inputs (Nunes et al. 2011, Idenyi et al. 2022). These ingredients have high nutritional value and high digestibility; however, they often come from extractive fisheries, which leads aquaculture, a sector in continuous development, to depend on detrimental fishing practices for the supply of necessary inputs (Amaya et al. 2007, Tacon & Metian 2008, Olsen & Hasan 2012, FAO 2020). In 2018, 12% of total fish production was used for non-food purposes, equivalent to 21.48 million tons. In recent years, a decrease in the use of fish oil has been observed, largely due to ethical and environmental issues and the increase in the price of this input, which has also been used as a nutritional supplement for human health. Furthermore, trends point to an even greater decrease in the coming decades, and it is believed that these ingredients will only be used strategically, at low levels, and in specific stages of development (FAO 2020).

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Therefore, to maintain the growth and sustainability of aquaculture, it is necessary to find ingredients that can replace fish oil and meal for use in aquafeed. Several studies demonstrate the possibility of replacing fish meals with ingredients of vegetable origin or by-products of animal origin (Amaya et al. 2007, Sá et al. 2013, Chen et al. 2015). However, some studies establish a direct relationship between the decrease in the use of fish meal and the increase in the use of fish oil so that shrimp growth is not negatively affected (Sá et al. 2013, Chen et al. 2023). Replacing fish oil is more complex and less studied, but new research has shown the possibility of replacing this dietary ingredient with vegetable and microalgae oils (González-Félix et al. 2010, Wang et al. 2017, Corrêa et al. 2018, Kumar et al. 2018, Perez-Velazquez et al. 2018, Allen et al. 2019, Guimarães et al. 2019, Tibbetts et al. 2020).

Aquatic animals have limited capacity for synthesizing long-chain polyunsaturated fatty acids (LC-PUFA) and must obtain them through their diets. When excessively replacing feed ingredients with vegetable sources (poor in n-3 and rich in n-6), the cultivated animal will have a lower content of these fatty acids in their meat because the content of fatty acids in the animal's muscle at the end of cultivation (the product delivered to the consumer) reflects the fatty acid content of their diet (Suárez-Mahecha et al. 2002, Martin et al. 2006, Amaya et al. 2007, Domingo et al. 2007, Turchini et al. 2009, González-Félix et al. 2010, NRC 2011, Allen et al. 2019, Guimarães et al. 2019). Plant-based foods like legumes, cereals, and vegetable oils are rich in n-6 polyunsaturated fatty acids and are already present in human food, coming from different sources. On the other hand, n-3 polyunsaturated fatty acids are present in fish and shrimp, serving as functional foods in the prevention of some diseases, being increasingly sought after by humans seeking a healthy diet and a better quality of life (González-Félix et al. 2010, Trautwein & McKay 2020, Idenyi et al. 2022, Langyan et al. 2022).

At the same time, alternative ingredients to fish meal and oil must be used in aquaculture. The food to be produced (in this case, shrimp) with the chosen inputs must be of high quality, meaning that these ingredients must be nutritionally rich, contain n-3 fatty acids, and be potentially rich in this class of fatty acids. In this context, microalgae-derived sources stand out, as they may contain high amounts of fatty acids (even long-chain ones) in their biomass (Richmond 2004, Idenyi et al. 2022, Chen et al. 2023). The use of microalgae for food production makes it possible to obtain healthy products with high nutritional value, bringing many benefits associated with n-3 series fatty acids for human health (Suárez-Mahecha et al. 2002, Martin et al. 2006, Amaya et al. 2007, Domingo et al. 2007, Turchini et al. 2009, NRC 2011, Guimarães et al. 2019). Furthermore, this ingredient makes it possible to maintain fish's most significant nutritional contribution: high-quality protein, micronutrients, and PUFAs (FAO 2020).

The microalgae *Aurantiochytrium* sp. contains high lipid concentrations (more than 70% of its biomass) and has a fatty acid profile with a high amount of docosahexaenoic acid (DHA) (Lewis et al. 1999). Similarly, microalgae of the genus *Nannochloropsis* are rich in lipids and contain high levels of eicosapentaenoic acid (EPA) (Volkman et al. 1993, Hulatt et al. 2017, Ashour et al. 2019). It has been proved that the use of these microalgae in shrimp feeding is linked to several benefits, such as improved growth, survival, and increased nutritional quality of the meat of the animals produced, with an increase in polyunsaturated fatty acids (Ju et al. 2009, Sánchez et al. 2014, Gamboa-Delgado & Márquez-Reyes 2018, Guimarães et al. 2019).

For farmed shrimp, several studies demonstrate that a balanced concentration of fatty acids in the diet promotes their growth (Glencross & Smith 1999, Gong et al. 2000, NRC 2011). Glencross & Smith (1999) demonstrated that maintaining the balance between linoleic and linolenic fatty acids increased animal growth. In 2001, they found that the combination of EPA and DHA promoted the same positive result. In another study, in which DHA, linoleic, and linolenic fatty acids were added to the Pacific white shrimp (*Penaeus vannamei*) diet, the authors reported the animals' growth and also an increase in their weight gain (González-Félix et al. 2002, 2003). Likewise, Guimarães et al. (2019) demonstrated that the total replacement of fish oil with DHA-rich *Aurantiochytrium* sp. meal was possible without negative effects on the performance parameters of the animals. Although the amount of n-3 fatty acids also increased with this microalga meal, an increase in saturated fatty acids (contained in the meal) was also observed, reflected in the shrimp meat.

This study evaluated the use of *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals to find a balanced meal composition replacing fish oil at five levels (0, 25, 50, 75, and 100%) regarding the use of DHA and EPA. The diet was formulated without a fish meal, and the Pacific white shrimp performance parameters and fatty acid profile were subsequently evaluated.

MATERIALS AND METHODS

The study was conducted at the Marine Shrimp Laboratory (LCM, by its Portuguese acronym) of the Federal University of Santa Catarina (UFSC, by its Portuguese acronym), Brazil. Marine shrimp species, *P. vannamei* of the Speedline Aqua lineage, from Aquatec Aquacultura Ltda. (Rio Grande do Norte, Brazil) were used. Postlarvae were kept in a biofloc system until they reached the appropriate size for the experiment - a mean weight of 3.37 ± 0.29 g.

Experimental diets

Aurantiochytrium sp. meal was acquired from Alltech - All-G-Rich (USA), and *Nannochloropsis* spp. meal from Necton-PhytoBloom (Portugal). Proximate composition analysis (Table 1) and feed preparation were conducted by the Fish Nutrition Laboratory (LABNUTRI, by its Portuguese acronym) of the Department of Aquaculture at the UFSC, Brazil, following standard procedures (AOAC 1999).

Five experimental diets were formulated with approximately 37.20% crude protein, 3757.86 kcal kg^{-1} of energy, and 8.63% of the ethereal extract, representing five levels of fish oil replacement (Table 2) by the *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals: 0% (control), 25, 50, 75 and 100%. The formulation was designed to maintain the DHA:EPA ratio between treatments, as fish oil was proportionally replaced by *Aurantiochytrium* sp. meal as a source of DHA and *Nannochloropsis* spp. meal as a source of EPA. In addition, ingredients were included in percentages to keep the experimental diets isoproteic and isoenergetic.

The diets were formulated using Optimal Formula 2000 based on nutritional recommendations and requirements for excellent performance in *P. vannamei*. For the unidentified requirements, the species *Penaeus monodon* was used as a reference (NRC 2011). Registration of the ingredients was based on analyses and reports of the company that provided the material in combination with bibliographic research (Akiyama 1988, Rostagno et al. 2005, Lima et al. 2006, 2011, Turchini et al. 2009).

Feed ingredients were first ground and then passed through a 600 μm sieve. Each diet had its ingredients weighed, separated and then mixed during preparation. First, the macro-ingredients were dry mixed, followed by adding the previously mixed micro-ingredients. Once the mixture was ready, oils and soy lecithin were added. Finally, moisture content was adjusted to 19%, and each diet was processed separately and pelletized (Inbramaq, MX-40) through a 1.5 mm matrix, resulting in a final pellet size of 2 mm. Pellets were dried overnight at 40°C until they reached a moisture content of 19% (approximately 1 h, with humidity, checked every 10 min). The feed was then kept frozen until feeding to avoid oxidation and loss of fatty acids from the diet.

Analysis of the diets

The diets were analyzed using the LABNUTRI and followed the Association of Official Analytical Chemists methodology (AOAC 1999). Diets were submitted to analyses of dry matter (drying at 105°C), ash (incineration at 550°C), protein (Kjeldahl, N×6.25), and ethereal extract (Soxleth after acid hydrolysis).

Fatty acid analyses were performed by gas chromatography using the Bligh & Dyer (1959) described by Corrêa et al. (2018). Menhaden and a mix of 37 oils were used to compare the retention times of fatty acids.

Analysis of shrimp muscle tissue

At the end of the experiment, muscle tissue was sampled from three shrimp per tank (n = 3), totaling nine shrimp per treatment, to analyze lipids and fatty acids. The analysis was conducted at the LABNUTRI, Department of Aquaculture, UFSC, Brazil. Briefly, shrimp muscle tissue lipids were extracted and quantified by the Folch et al. method (1957), as modified by Ways & Hanahan (1964). The fatty acids were then esterified using the method of O'Fallon et al. (2007) and separated by a gas chromatograph equipped with a capillary column. The carrier gas was nitrogen, and the forming gases were nitrogen and hydrogen. The operational parameters were those performed by Nobrega et al. (2017).

Experimental design and conditions

A completely randomized design with three replicates per treatment, resulting in 15 experimental units, was used to evaluate five replacement levels (0, 25, 50, 75, and 100%) of fish oil by *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals. Experimental units were circular 500 L polyethylene flat-bottom tanks filled with 400 L of seawater and provided with aeration, thermostats, and heaters. Each experimental unit was stocked with 40 shrimp with an average weight of 3.37 ± 0.29 g, resulting in an initial stocking density of 60 shrimp m^{-3}.

All tanks were filled with seawater from Barra da Lagoa Beach (Florianópolis, SC, Brazil), with a salinity of 31.74 and alkalinity of 132.8 mg L^{-1}, pH 8, ammonia

Table 1. Summary of nutritional information and proximate composition of fish oil, *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals, detailing their most representative fatty acids. *Data provided by the manufacturer. **Analysis was performed by the Fish Nutrition Laboratory of the Department of Aquaculture at UFSC, following standard procedure (AOAC 1999). [1]Fish oil distributed by BFP Bio Food Products (Itajaí, Santa Catarina, Brazil).

Fish oil[1]			
Gross energy*	8,445.00 kcal kg^{-1}	Vitamin A*	300.00 mg kg^{-1}
Ethereal extract*	99.00%	Vitamin D*	2500.00 mg kg^{-1}
Cholesterol*	0.57%	Vitamin E*	220.00 mg kg^{-1}
Fatty acids** (% lipids):			
Myristic (14:0)	6.53	Erucic (22:1 n-9)	0.52
Palmitic (16:0)	12.99	Linoleic (18:2 n-6)	0.86
Stearic (18:0)	2.41	Stearidonic (18:4 n-3)	1.77
Palmitoleic (16:1 n-7)	7.13	Arachidonic (20:4 n-6)	1.44
cis-Vaccenic (18:1 n-7)	2.52	α-linolenic (18:3 n-3)	0.69
Oleic (18:1 n-9)	4.44	Eicosapentaenoic (20:5 n-3)	12.84
Gondoic (20:1 n-9)	0.48	Docosahexaenoic (22:6 n-3)	5.42
Aurantiochytrium sp. meal (g 100 g^{-1} dry matter)			
Moisture**	1.29	Fatty acids** (% lipids):	
Crude fat**	60.43	Myristic (14:0)	2.03
Crude fiber*	0.90	Palmitic (16:0)	23.08
Carbohydrates*	24.88	Stearic (18:0)	0.68
Proteins**	11.26	Eicosapentaenoic-EPA (20:5 n-3)	0.13
Mineral*	3.67	Docosahexaenoic-DHA (22:6 n-3)	10.85
Nannochloropsis spp. meal (g 100 g^{-1} dry matter)			
Moisture**	2.84	Fatty acids** (% lipids):	
Crude fat**	18.95	Myristic (14:0)	0.57
Crude fiber*	0.90	Palmitic (16:0)	2.80
Carbohydrates*	24.88	Oleic (18:1 n-9)	0.86
Proteins**	42.85	Eicosapentaenoic-EPA (20:5 n-3)	2.97
Mineral*	3.67	Docosahexaenoic-DHA (22:6 n-3)	0.10

of 0.3 mg L^{-1}; and nitrite of 0 mg L^{-1}. During the experimental period, water quality indicators were monitored periodically. Alkalinity was analyzed using the APHA (1995) method, and the analysis of ammonium and nitrite followed the method of Strickland & Parsons (1972). Variables, including dissolved oxygen, temperature, pH, and salinity, were measured with a multi-parameter YSI-Professional Plus meter. During the experiment, dissolved oxygen (6.87 ± 0.02 mg L^{-1}), temperature (28.43 ± 0.04°C), pH (8.07 ± 0.00), alkalinity (127.94 ± 2.70 mg L^{-1}) and salinity (32.92 ± 0.13) remained stable; and ammonia (<0.5 mg L^{-1}), and nitrite (<0.14 mg L^{-1}) at low levels, within stipulated limits and suitable for marine shrimp (Boyd & Gautier 2000).

According to the table developed by Van Wyk et al. (1999), feed was initially supplied at 7% of tank biomass per day. This amount was adjusted weekly based on shrimp weight and feed consumption. During the weekly biometrics, all animals were removed from the tanks, counted, and weighed, and the next feeding was adjusted accordingly (Davis et al. 2004).

Feed was provided four times a day (08:30, 12:00, 13:30, and 17:30 h) using feed trays (0.03 m^2), and consumption was checked 1.5 h after the feed was offered to verify consumption. Water was exchanged daily in the afternoon at 80% of the total tank volume with total removal of the remaining organic matter, including feces, feed, and molts.

Performance parameters

The experimental period lasted 49 days, and the final performance parameters (zootechnical parameters) of shrimp were calculated as follows:

- Survival (%) = (final number of shrimp / initial number of shrimp) × 100
- Weekly weight gain (g $week^{-1}$) = {[final average weight (g) – initial average weight (g)] / days of cultivation} × 7
- Feed conversion rate = feed given (g) / weight gain (g)

Table 2. Formulation of experimental diets containing five different levels of microalgae meal (*Aurantiochytrium* sp. and *Nannochloropsis* spp.) as a replacement for fish oil and their respective proximal composition. [a]In Vivo Nutrição e Saúde Animal Ltda. (São Paulo, SP, Brazil): vit. A: 900 mg kg^{-1}; vit. D_3: 25 mg kg^{-1}; vit. E: 46,900 mg kg^{-1}; vit. K_3: 1400 mg kg^{-1}; cobalamin (B12): 50 mg kg^{-1}; piridoxine (B6): 33,000 mg kg^{-1}; riboflavina: 20,000 mg kg^{-1}; nicotinic acid: 70,000 mg kg^{-1}; pantothenic acid: 40,000 mg kg-$^{-1}$; biotin: 750 mg kg^{-1}; folic acid: 3000 mg kg^{-1}; copper: 2,330 mg kg^{-1}; zinc: 10,000 mg kg-$^{-1}$; manganese: 6500 mg kg^{-1}; selenium: 125 mg kg^{-1}; iodine: 1000 mg kg^{-1}; cobalto: 50 mg kg^{-1}; magnesium: 20 g kg^{-1}; potassium: 6.1 g kg^{-1}. [b]L-ascorbic acid-2-monophosphate 35%. DSM Nutritional Products Brazil (São Paulo, SP, Brazil). *Analysis performed by the Fish Nutrition Laboratory of the Department of Aquaculture at UFSC, following standard procedure (AOAC 1999): dry matter by method 950.01; mineral matter by method 942.05; protein by Kjeldahl, conversion factor 6.25; ethereal extract by Soxhlet by method 920.39C. Gross energy was determined using a bomb calorimeter, and fatty acid analysis was done by Bligh-Dyer (1959).

Ingredients (g 100 g^{-1} dry diet)	Replacement (%)				
	0	25	50	75	100
Soybean meal	33.00	33.00	33.00	33.00	33.00
Soybean protein concentrate	16.05	13.20	10.40	7.50	5.00
Wheat meal	13.50	14.00	14.50	15.00	15.00
Poultry by-product meal	12.00	12.00	12.00	12.00	12.00
Kaolin	9.60	8.52	7.37	6.34	5.59
Soy lecithin	2.20	2.20	2.20	2.20	2.20
Monocalcium phosphate	3.00	3.00	3.00	3.00	3.00
Magnesium sulfate	1.50	1.50	1.50	1.50	1.50
Sodium chloride	1.50	1.50	1.50	1.50	1.50
Mineral premix[a]	1.64	1.64	1.64	1.64	1.64
Calcium chloride	1.00	1.00	1.00	1.00	1.00
Carboxymethylcellulose	0.50	0.50	0.50	0.50	0.50
Vitamin premix[a]	0.36	0.36	0.36	0.36	0.36
Choline hydrochloride	0.10	0.10	0.10	0.10	0.10
Vitamin C[b]	0.03	0.03	0.03	0.03	0.03
Purified cholesterol	0.02	0.02	0.03	0.03	0.04
Methionine	0.00	0.03	0.07	0.10	0.14
Fish oil	4.00	3.00	2.00	1.00	0.00
Aurantiochytrium sp. meal	0.00	0.40	0.80	1.20	1.70
Nannochloropsis spp. meal	0.00	4.00	8.00	12.00	15.70
Proximal composition*					
Total protein	38.13	37.33	38.37	37.93	37.64
Gross energy (kcal kg^{-1})	3910.41	3995.80	4037.94	4109.59	4138.46
Moisture	7.88	5.36	5.64	5.61	5.08
Ash	18.88	18.37	17.54	16.90	16.40
Ethereal extract	7.52	8.42	8.16	8.42	8.56

Statistical analysis

Statistical analysis was performed with Statistica 10 (StatSoft®) software, using linear and quadratic regression and a 5% significance level.

RESULTS

Performance parameters

The shrimp presented standard growth performance, reaching 1.89 g $week^{-1}$ on average, with a feed conversion of around 1.47, survival above 97% between treatments, and a final weight of 16.59 g. No significant difference was observed in the growth performance parameters analyzed (Table 3).

Analysis of the diets

In the formulated diets, the percentage of fatty acid was very similar among all treatments, including DHA and EPA had few changes with the inclusion of microalgae meal (Table 4).

Analysis of muscle fatty acids profile

The fatty acid profile in the animals' muscles was not significantly different among treatments. The amounts

Table 3. Performance of shrimp *Penaeus vannamei* fed diets with different levels of fish oil replacement by *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals for 49 days. NS: not significant.

Replacement (%)	Final weight (g)	Weekly weight gain (g week^{-1})	Feed conversion	Survival (%)
0	16.25 ± 0.47	1.84 ± 0.32	1.50 ± 0.25	97.50
25	16.57 ± 0.55	1.88 ± 0.36	1.48 ± 0.33	97.50
50	16.69 ± 0.23	1.90 ± 0.31	1.46 ± 0.29	97.50
75	16.74 ± 0.09	1.91 ± 0.22	1.44 ± 0.26	98.33
100	16.70 ± 0.59	1.90 ± 0.31	1.46 ± 0.27	98.33
Quadratic regression analysis	NS	NS	NS	NS

Table 4. Fatty acid profile in diets containing increasing levels of *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals to replace fish oil. ND: not detected (values <0.01% of total fatty acid). [a]Fatty acid groups: SFA: saturated, MUFA: monounsaturated, PUFA: polyunsaturated.

Fatty acid profile in diets	Replacement (%)				
Fatty acids (% dry matter)	0	25	50	75	100
Myristic (14:0)	0.22	0.21	0.18	0.16	0.09
Pentadecanoic (15:0)	0.01	0.01	0.01	0.02	0.02
Palmitic (16:0)	0.87	1.05	1.12	1.33	1.12
Palmitoleic (16:1 n-7)	0.24	0.17	0.27	0.32	0.28
Hexadecatrienoic (16:3 n-4)	0.03	ND	0.01	ND	0.56
Margaric (17:0)	0.01	0.01	0.01	0.01	0.01
Heptadecanoic (17:1 n-7)	0.02	0.01	ND	ND	ND
Stearic (18:0)	0.20	0.21	0.18	0.18	0.12
Oleic (18:1 n-9)	0.64	0.40	0.56	0.57	0.48
Vaccenic (18:1 n-7)	0.13	0.07	0.08	0.07	0.05
Linoleic (18:2 n-6)	0.63	0.62	0.65	0.66	0.65
α-linolenic (18:3 n-3)	0.02	0.01	0.01	0.01	ND
Eicosadienoic (20:2 n-6)	0.01	0.06	0.03	0.04	0.02
Eicosatrienoic (20:3 n-3)	ND	0.01	0.04	0.05	0.05
Eicosapentaenoic-EPA (20:5 n-3)	0.15	0.16	0.16	0.16	0.14
Behenic (22:0)	ND	0.01	0.01	0.01	ND
Docosahexaenoic-DHA (22:6 n-3)	0.05	0.04	0.06	0.05	0.07
Docosadienoic (22:2 n-6)	0.03	0.03	0.03	0.05	ND
Tricosanoic (23:0)	0.16	0.18	0.18	0.16	0.15
Lignoceric (24:0)	0.02	0.03	0.02	0.02	ND
Groups of fatty acids[a]					
SFA	1.51	1.73	1.73	1.90	1.51
MUFA	1.09	0.66	0.96	1.00	0.84
PUFA	0.97	0.93	1.00	1.03	1.51
PUFA n-3	0.23	0.22	0.28	0.28	0.26
PUFA n-6	0.69	0.70	0.70	0.75	0.68
n-3/n-6	0.33	0.31	0.40	0.37	0.38

and proportions of n-3 and n-6 remained constant regardless of substitution levels (Table 5).

DISCUSSION

The formulated diets presented similar protein, energy, lipid, and DHA:EPA ratio levels, meeting the nutritional requirements of the species *Penaeus vannamei* (NRC 2011). The proper formulation of the diets was reflected in the growth of the animals, which grew more than 1.84 g per week, a higher value than that reported by other studies with the same species in the grow-out phase (Suárez et al. 2009, Bauer et al. 2012).

Table 5. Fatty acids profile in shrimp (*Penaeus vannamei*) after 49 days of feeding with diets containing increasing levels of *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals to replace fish oil. *Linear and quadratic regression tests were performed. NS: not significant. [a]Fatty acid groups: SFA: saturated, MUFA: monounsaturated, PUFA: polyunsaturated. **Saturated fatty acids.

Shrimp muscle fatty acid profile	Replacement (%)					
Fatty acids (% dry matter)	0	25	50	75	100	Regression*
Palmitic (16:0)	0.31	0.35	0.37	0.41	0.36	NS
Stearic (18:0)	0.15	0.11	0.18	0.18	0.17	NS
Oleic (18:1 n-9)	0.20	0.21	0.21	0.23	0.19	NS
Linoleic (18:2 n-6)	0.14	0.15	0.21	0.24	0.14	NS
α-linolenic (18:3 n-3)	0.01	0.01	0.01	0.02	0.02	NS
Eicosatrienoic (20:3 n-3)	0.05	0.06	0.07	0.09	0.08	NS
Trichosanoic (23:0)	0.09	0.10	0.09	0.10	0.09	NS
Eicosapentaenoic-EPA (20:5 n-3)	0.24	0.24	0.23	0.23	0.18	NS
Docosahexaenoic-DHA (22:6 n-3)	0.12	0.15	0.14	0.16	0.15	NS
Groups of fatty acids[a]:						
Others**	0.12	0.13	0.10	0.12	0.07	NS
SFA	0.57	0.59	0.67	0.72	0.65	NS
MUFA	0.27	0.34	0.30	0.31	0.26	NS
PUFA	0.64	0.71	0.70	0.75	0.61	NS
PUFA n-3	0.43	0.47	0.46	0.49	0.39	NS
PUFA n-6	0.17	0.18	0.22	0.26	0.18	NS
n-3/n-6	2.52	2.61	2.09	1.88	2.16	NS

Survival remained high, with no significant difference between treatments for this and also for the other performance parameters, similar to what was observed in other studies with the replacement of fish oil meeting the nutritional requirements of the species (González-Félix et al. 2010, Allen et al. 2019). Moreover, the total replacement of fish oil did not compromise these parameters since they presented similar values between the control and the treatment with 100% replacement, which corroborates with Chen et al. (2015), who demonstrated that an adequate proportion of fatty acids in diets with replacement of fish oil could promote growth and survival equal to that obtained by diets with fish oil.

Studies show that the muscle composition of shrimp - the final meat composition, directly reflects the composition of the González-Félix et al. (2010) diet when replacing fish oil with soybean and linseed oil, observed a decrease in n-3 series fatty acids, whereas Guimarães et al. (2019), when replacing fish oil with *Aurantiochytrium* sp. meal, observed, according to the inclusion levels, an increase in some fatty acids, such as palmitic acid. In this study, unlike those mentioned, a balanced concentration of fatty acids in the muscles of the animals was observed. As the substitutions were made, the amounts and proportions were also maintained. Even when comparing the percentage of EPA and DHA found in the muscles of animals fed with microalgae meal, it was slightly higher when compared to the percentage found in the diets. This interesting result can help to obtain the ideal composition of a microalgae oil that replaces fish oil and generates a final product with the nutritional quality desired by the consumer.

Finally, we emphasize that the diet with 100% replacement of fish oil by microalgae meal provided excellent performance results, indicating that it is possible to formulate a high-performance diet for shrimp without any ingredients of marine origin, either fish oil or meal, using only poultry by-product meal as the sole ingredient of animal origin. Thus, we demonstrate that replacing fish oil with microalgae meal is feasible, contrary to what has been reported by several studies (Amaya et al. 2007, Sá et al. 2013). It is known that although the production and acquisition of microalgae meals are more expensive than obtaining fish oil, the latter is a scarce source that will probably no longer be used shortly (Sprague et al. 2017, FAO 2014, 2020), which is why we believe that, more and more incentives will be given to the use of this

promising raw material to contribute to the sustainability of aquaculture. It is still expensive to use full replacement by microalgae, but soon, it will be possible with other alternative sources for proteins and oils.

Therefore, the present data suggests that it is possible to replace fish oil with a product that does not require extractive fishing, maintaining the nutritional quality of the meat, such as protein, micronutrients, and n-3 and n-6 levels, which are, of course, important to human health.

CONCLUSIONS

Partial or total replacement of fish oil by *Aurantiochytrium* sp. and *Nannochloropsis* spp. meals did not affect shrimp performance and maintained the fatty acid composition of shrimp muscle.

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