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ADDITION OF *Aurantiochytrium* sp. MEAL IN THE DIET AFFECTS IMMUNITY AND THERMAL SHOCK RESISTANCE OF THE PACIFIC WHITE SHRIMP*

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ABSTRACT

This paper reports the evaluation of digestibility, immunological parameters, and resistance to thermal shock at low temperature in *Litopenaeus vannamei* fed diets with diferent *Aurantiochytrium* sp. meal additions (0; 0.5; 1 and 2%). First, the apparent digestibility coefficient of the ingredient was determined. The digestibility of the microalgae meal was high for protein (74.90%); around 60% for lipids, and for docosahexaenoic fatty acid (DHA) it was 55.61%. After, shrimp rearing with the feed additive was carried out in a clear water system, containing 25 shrimp (initial weight: 4.89 ± 0.27g) per 400 L tank. Feeding occurred four times a day. After a three-week period, immunological parameters were evaluated and thermal shock was performed. Animals fed 0.5% and 2% of the microalgae *Aurantiochytrium* sp. showed higher survival to thermal shock. In immunological analyses, the serum agglutiniting titer was higher (p <0.05) in the 0.5 and 2% additions of the microalgae meal, and the phenoloxidase activity (PO) was higher in the 1% addition (p <0.05). It is concluded that there is good utilization of the nutrients of *Aurantiochytrium* sp. meal by *L. vannamei* and its addition to the diet (0.5 and 1%) increased shrimp resistance to thermal shock.

Key words: *Litopenaeus vannamei*; DHA; immunity; microalgae; temperature.

ADIÇÃO DE FARINHA DE *Aurantiochytrium* sp. NA DIETA AFETA A IMUNIDADE E RESISTÊNCIA AO CHOQUE TÉRMICO DO CAMARÃO-BRANCO-DO-PACÍFICO

RESUMO

Avaliou-se a digestibilidade, os parâmetros imunológicos e a resistência ao choque térmico a baixa temperatura em *Litopenaeus vannamei* alimentado com dietas contendo diferentes proporções de farinha da microalga *Aurantiochytrium* sp. (0; 0,5; 1 e 2%). Inicialmente, foi determinado o coeficiente de digestibilidade aparente do ingrediente. A digestibilidade da farinha da microalga foi alta para proteína (74,9%), e em torno no 60% para lipídeos em geral, sendo que para o ácido graxo docosahexaenóico (DHA) foi de 55,61%. Posteriormente, o cultivo com o aditivo alimentar foi realizado em sistema de água clara, contendo 25 camarões (peso inicial 4,89 ± 0,27 g) por tanque de 400 L, alimentados quatro vezes ao dia. Após o período de três semanas, foram avaliados os parâmetros imunológicos e realizado o choque térmico. Os animais alimentados com 0,5% e 1% da microalga na dieta apresentaram melhor sobrevivência ao choque térmico. Nas análises imunológicas, o título aglutinante do soro foi significativamente superior (p <0,05) nas adições de 0,5 e 2% da farinha da microalga, e a atividade da fonoloxidade (PO) na adição de 1% (p <0,05). Conclui-se que a farinha de *Aurantiochytrium* sp. tem bom aproveitamento de seus nutrientes por *L. vannamei* e sua adição na dieta (0,5 e 1%) aumentou a resistência dos camarões à variação térmica.

Palavras-chave: *Litopenaeus vannamei;* DHA; imunidade; microalga; temperatura.

INTRODUCTION

Brazilian aquaculture has advanced with the application of methods aimed at sustainability and this has contributed to the growth of food production of animal origin (FAO, 2016). In this context, marine shrimp farming is among the most important sectors of Brazilian aquaculture, with *Litopennaeus vannamei* highlighted, not only for

its market value, but also for its adaptability to the most varied growing conditions (FAO, 2016).

However, the development of national shrimp farming has been hindered by factors such as poor management practices at some farms, the provision of inadequate diets and the intensification of rearing (Lightner, 2011). In addition, natural changes in the environment, such as in temperature, contributes to immune suppression, thus favoring outbreaks of diseases, affecting the development of the sector (Kautsky et al., 2000; Moser et al., 2012; Thanigaivel et al., 2016).

Thermal variation represents a genuine challenge for aquatic organisms, as there are ranges of environmental conditions to which they are best adapted, and an optimal range for physiological and ecological performance (Willmer et al., 2005). For *L. vannamei*, the optimal range is between 28 and 32ºC (Van Wyk and Scarpa, 1999).

When an increase or decrease in temperature occurs, the shrimp organism reacts, causing a chain of genes to be regulated and promoting a readjustment of the physiological processes (homeostasis) necessary to maintain a stable internal environment (Yenari et al., 1999). All of these factors are stressful to the animal and serve as a trigger for the occurrence of diseases. There are methods in use to increase the immunocompetence of animals, such as the use of immunostimulants in the diet (Shah et al., 2018).

The use of compounds capable of increasing immunity in shrimp has gained special attention for successfully promoting immune protection in crustaceans exposed to pathogens experimentally (Teunissen et al., 1998; Alabi et al., 1999; Schleder et al., 2017; Alves Da Silva et al., 2020). One of the compounds used is derived from microalgae, which has been widely applied in aquaculture as a feed additive. Microalgae contain several substances such as polysaccharides and vitamins, as well as elongases and desaturases that are responsible for the production of long-chain fatty acids of groups n-3 and n-6 (Harwood and Guschina, 2009; Ju et al., 2012).

In this context, the incorporation of seaweed in shrimp diets has shown to be promising (Xia et al., 2012; Niu et al., 2015). The use of artemia biomass and the microalgae *Spirulina platensis* as a feed supplement for Pacific white shrimp post-larvae resulted in an increase in weight and length, and high survival rate (Neto et al., 2008). Dry *Arthrospira* can be replaced by fishmeal up to 75% in a typical shrimp diet, without affecting survival and growth. In addition to improving the immune response, it promotes an increase in the number of granular hemocytes and a reduction in apoptosis in *L. vannamei* (Macias-Sancho et al., 2014).

Among microalgae, *Aurantiochytrium* sp. has been recognized for its high capacity for lipid production, especially docosahexaenoic fatty acid (DHA). This species is classified as an oleaginous microorganism due to its lipid production potential (Chi et al., 2009). Guimarães et al. (2019), observed that the total substitution of fish oil (cod liver) with meal from *Aurantiochytrium* sp. added DHA to *L. vannamei* muscle. Wang et al. (2017) obtained similar results, showing that fish oil can be replaced at up to 150 g kg^{-1} by *Aurantiochytrium* sp. (ALLTECH SP1) in the feeding of larvae of *L. vannamei*.

The authors further suggest that *Aurantiochytrium* sp. can be used as an alternative source of essential fatty acids, in particular DHA, in diets of *L. vannnamei* larvae. The presence of these compounds acts as an immunostimulant in fish and crustaceans, providing greater resistance to bacterial pathogens, viral infections, and stressful situations (Sakai, 1999; Zhou et al., 2012; Li et al., 2013). In this context, the objective of the present study was to evaluate the influence of *Aurantiochytrium* sp. as a feed additive for *L. vannamei* species, focusing on digestibility, immune responses (parameters) and resistance to thermal shock.

MATERIAL AND METHODS

Biological material

The shrimps used were of the species *Litopenaeus vannamei*, acquired from the company Aquatec Aquacultura Ltda (RN), lineage SPEEDLINE HB12.

Formulation of diets

The diets were formulated using the Optimal Formula 2000® program version 19102009, based on the recommendations and nutritional requirements for the optimal performance of the Pacific white shrimp (Gong et al., 2000; NRC, 2011; Zhou et al., 2012). Two diets were formulated, one for the digestibility test and another for the experiment as a feed additive to verify its effects on the shrimp organism, both using the microalgae meal of the species *Aurantiochytrium* sp. (Table 1).

For the digestibility test, a reference diet was formulated with only semi-purified ingredients (Table 2), where 1.00 mg g^{-1} of the dry yttrium oxide diet was added as an inert marker. The test diet contained 900 mg g^{-1} dry weight of the reference diet and 100 mg g^{-1} dry weight of *Aurantiochytrium* sp. (Table 2). For the feed additive trial, the diets were formulated with practical ingredients, with three inclusions do *Aurantiochytrium* sp. meal (0.5, 1 and 2%) and a control with no inclusion (Table 3).

For both diets, the feed ingredients were first ground and sieved in 600 µm mesh, then mixed dry, all the macro ingredients and then micro ingredients. After each mixture was ready, soy oils and lecithin were added. Finally, humidity was adjusted to 15%. The diet was pelleted in a 1.5 mm matrix, and kiln dried at 40°C for approximately 12 h. Then, the feed was kept frozen until the moment of each feeding, to avoid oxidation and loss of fatty acids from the diets.

Analysis of diets

The analysis of the diets was carried out by the Aquaculture Species Nutrition Laboratory (LabNutri), following the methodology described by Association of Official Analytical Chemists (AOAC, 1999). The diets were subjected to dry matter analysis with drying at 105°C, ash with burning at 550°C, protein by Kjeladahl (N x 6.25), and ether extract by Soxleth after acid hydrolysis. Fatty acid analyses were measured by gas chromatography using the modified method of Folch et al. (1957) as described by Corrêa et al. (2018).

Table 1. Composition of *Aurantiochytrium* sp. (g 100 g^{-1} dry matter) detailing its most representative fatty acids.

Nutrient[a]	*Aurantiochytrium* sp. (g 100 g^{-1} dry matter)
Dry matter	97.16
Moisture	3.70
Total fiber	0.90
Carbohydrates	24.88
Proteins	19.22
Minerals	3.67
Total fat	50.00
Fatty Acids[a]	
14:0 Myristic acid	2.04
16:0 Palmitic acid	23.28
18:0 Stearic acid	0.67
18:1 n-9 Oleic acid	0.03
18:2 n-6 Linoleic acid	0.01
18:3 n-3 Linolenic acid	0.10
20:4 n-6 Arachidonic	0.03
20:5 n-3 EPA	0.13
22:6 n-3 DHA	10.83
SFA[b]	27.38
MUFA	0.39
PUFA	13.83
LC-PUFA n-6	2.63
LC-PUFA n-3	11.18

[a] Analysis conducted at the Aquaculture Species Nutrition Laboratory - LABNUTRI/UFSC. [b] Fatty acid groups: SFA = saturated, MUFA = monosaturated, PUFA = polyunsaturated.

Digestibility Test

For the digestibility test, 400 shrimp of 8.00 ± 0.30 g were used, stored in two 5000 L tanks in a clear water system (70% of water exchange per day). They were fed four times a day (8:00 am, 12:00 pm, 2:00 pm, 6:00 pm) and acclimatized with the diets for ten days. After this period, faeces collections began, where 10 animals from each treatment were transferred to 60 L aquariums. Collections were made in quadruplicate for each treatment, in a total of eight experimental units, in a room equipped with an aeration system (O_2 >5.0 mg L^{-1}) and heating (28.0 ± 1.0°C).

The animals were fed at 8 am in the morning and at 2 pm in the afternoon and after each feeding, 40 minutes was allowed to consume the feed. Thereafter, the aquariums were siphoned, for the removal of feces and leftover feed and the collection of feces began, lasting 4 hours. The aquariums were checked continuously to remove fresh feces, avoiding the degradation of the material. Feces were collected by siphoning with a plastic Pausteur pipette and placed in petri dishes, where they were gently and quickly washed with distilled water and transferred to falcon tubes, which were cooled with ice to prevent the degradation of fatty acids.

At the end of the day, water of the aquariums was changed at 90%; and the falcons were centrifuged twice at 1,800 g at 4°C for 10 min (model 5804R Eppendorf AG, Hamburg, Germany), to remove excess water, and frozen in a vertical freezer at -18°C. Repeat analyses were conducted by collection over time, with a final weight of the falcon tube approximately 80 g wet weight of feces.

Table 2. Formulation of the diets for the digestibility test and centesimal composition.

Ingredients (g kg^{-1} dry feed)	Reference Diet	Test Diet *Aurantiochytrium* sp.[h]
Casein[a]	256.30	230.67
Cornstarch[a]	223.90	201.51
Gelatin[a]	170.30	153.27
Aurantiochytrium sp. meal[c]	0.00	100.00
Kaolin[a]	114.00	102.60
Cellulose[a]	53.04	47.74
Lecithin	50.12	45.11
Magnesium sulfate	25.20	22.68
Cod liver oil	20.00	18.00
Potassium chloride	16.40	14.76
Mineral premix[d]	16.20	14.58
Soybean oil	16.00	14.40
Sodium chloride	14.40	12.96
Mono calcium phosphate	13.30	11.97
Carboxymethylcellulose[a]	4.24	3.82
Vitamin premix[d]	3.80	3.42
Coline hydrochloride	1.00	0.90
Yttrium oxide	1.00	0.90
Vitamin C[e]	0.80	0.72
Composition (g 100 g^{-1} dry matter)[f]		
Dry matter	93.65	95.07
Protein	41.07	40.62
Lipids	7.48	14.03
SFA[g]	1.48	5.64
MUFA	1.83	1.68
PUFA	3.71	4.98
LC-PUFA n-6	2.29	2.51
LC-PUFA n-3	0.88	2.47
n-3/n-6	0.39	0.98

[a] Distributed by Rhoster (Araçoiaba da Serra, São Paulo, Brazil). [b] All - G - Rich, produced by Alltech Inc. (Nicholasville, Kentuchy, USA), imported by Alltech do Brasil Agroindustrial Ltda (Araucária, Paraná, Brazil). [c] Produced by Alltech - All G Rich. [d] In Vivo Nutrição e Saúde Animal Ltda. (São Paulo, SP, Brazil): vit. A: 900 mg kg^{-1}; vit. D3: 25 mg kg^{-1}; vit. E: 46,900 mg kg^{-1}; vit. K3: 1,400 mg kg^{-1}; cobalamin (B12): 50 mg kg^{-1}; pyridoxine (B6): 33,000 mg kg^{-1}; riboflavin: 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: 3,000 mg kg^{-1}; copper: 2,330 mg kg^{-1}; zinc: 10,000 mg kg^{-1}; manganese: 6,500 mg kg^{-1}; selenium: 125 mg kg^{-1}; iodine: 1,000 mg kg^{-1}; cobalt: 50 mg kg^{-1}; magnesium: 20 g kg^{-1}; potassium: 6.1 g kg^{-1}. [e] L-ascorbic acid-2-monophosphate 35%. DSM Nutritional Products Brazil (São Paulo, SP, Brazil). [f] Analysis performed at the Aquaculture Species Nutrition Laboratory, LABNUTRI/UFSC. [g] Fatty acid groups: SFA = saturated, MUFA = monosaturated, PUFA = polyunsaturated. [h] Test diet contained 900 mg g^{-1} dry weight of the reference diet and 100 mg g^{-1} dry weight of *Aurantiochytrium* sp.

Table 3. Ingredients used in the formulation of the diets and the percentage composition of the experimental diets with microalgae *Aurantiochytrium* sp. meal supplementation.

Ingredients (g kg^{-1})	Control	0.5%	1%	2%
Soybean meal	450.0	450.0	450.0	450.0
Salmon residue meal	190.0	190.0	190.0	190.0
Wheat flour	150.0	150.0	150.0	150.0
Kaolin	90.0	85	80	70
Soy lecithin	30.0	30.0	30.0	30.0
Monocalcium phosphate	20.0	20.0	20.0	20.0
Sodium chloride	15.0	15.0	15.0	15.0
Magnesium sulphate	15.0	15.0	15.0	15.0
Mineral premix[1]	13.5	13.5	13.5	13.5
Cod liver oil	10.8	10.8	10.8	10.8
Soya oil	10.0	10.0	10.0	10.0
Carboximethyl cellulose	5.0	5.0	5.0	5.0
Vitamin C	0.7	0.7	0.7	0.7
Aurantiochytrium sp. meal	0	5	10	20
Percentage Composition (%)				
Moisture	9.4	9.3	9.3	9.4
Total protein	43.7	43.2	43.9	44.0
Ash	18.7	18.6	18.3	18.0
Ethereal extract	8.7	8.7	8.8	8.9
Total fibre	4.4	4.3	4.4	4.3

At the end of the collections, all falcons with feces were lyophilized, homogenized and the samples were separated for analysis of yttrium, crude protein, dry matter and lipids. The apparent digestibility coefficient of protein, dry matter and lipids was calculated as follows:

$$\%ADCn = 100 - \left[100 \text{ x} \left(\frac{\%\text{ C diet}}{\%\text{ C faeces}}\right) \text{x} \left(\frac{\%\text{ N faeces}}{\%\text{ N diet}}\right)\right]; \quad (1)$$

where C is the value of yttrium oxide in the diet or feces and N is the concentration of the nutrient considered (dry matter, protein, lipids, fatty acids) in the diet or feces (in% of dry matter).

The apparent digestibility coefficient of the constituent for dry matter, protein and lipids was calculated using the following equation:

$$\%ADCingr = \text{ADCtest diet} + \left[\left(\text{ADC test diet} - \text{ADC referenc diet}\right) \text{x} \frac{0.85 \text{ x N ref}}{0.15 \text{ x N ing}}\right]; \quad (2)$$

where ADC test diet and ADC reference diet are the apparent digestibility coefficients of the test diet and reference diet, calculated using the first formula, and N (ref and ing) are the concentrations of what is being calculated (dry matter, protein, lipids, acids fatty acids) present in the reference diet or in the ingredient.

Feed additive test

The *Aurantiochytrium* sp. as a feed additive experiment was conducted for 3 weeks in 12 circular polyethylene tanks (volume of 400 L) with water exchange (70% of volume) carried out once a day, always in the morning before the first feeding, with total removal of the remains of organic matter (feces, feed scraps and seedlings) so as not to compromise the water quality of the system. The experimental units were populated at a density of 25 shrimp per tank (average weight of 4.89 ± 0.27 g).

The experimental diets with different additions of *Aurantiochytrium* sp. (0; 0.5; 1 and 2%), were offered four times a day (8:30 am; 12:00 pm, 2:30 pm, 5:00 pm) in feed trays for later checking of consumption (90 minutes after food supply). The feed was supplied following the table proposed by Van Wyk and Scarpa (1999), where the animals were fed with the equivalent of 6% of the biomass of the tanks and the amount was adjusted weekly after the biometrics were performed. In the weekly biometrics, the animals were removed from the cultivation tanks to check survival and weigh together all the shrimp per tank.

During the experiment, the following factors were controlled: dissolved oxygen (5.6 ± 0.2 mg L^{-1}), and water temperature (29 ± 1°C) measured twice a day (08:30 am, and 4:30 pm) using an oximeter (YSI 5908®). Once a week, analysis of pH (8.3 ± 1.0) (pHmeter YSI 100®); salinity (35 ± 0 ppm) (Instrutherm®), alkalinity (>120 mg L^{-1}), ammonia (<1 mg L^{-1}); and nitrite (<0.01 mg L^{-1}) was carried out, according to Strickland and Parsons (1972).

Immunological analyses

To obtain serum for each treatment, the hemolymph of the shrimp was collected using a syringe (1 mL) with a needle (13 x 0.4 mm), inserted in the ventral region of the first abdominal segment of the shrimp. Three pools with four animals each were made for each analyzed group. For the preparation of the serum, the hemolymph

was allowed to clot for 2 hours at room temperature. The clot was broken with the aid of a glass stick and repeatedly centrifuged at 6,000 g for 10 minutes. The supernatant corresponding to the serum (containing plasma and cellular factors) was removed and frozen at –20°C for later use in assays of protein concentration and phenoloxidase activity.

The total hemocyte count (THC) were estimated in using a Neubauer chamber. Hemolymph (3 pools of 5 animals per group) was collected in fixative composed of 4% formaldehyde in anticoagulant solution MAS (Modified Alsever Solution) (336 mMNaCl, 115 mM glucose, 27 mM sodium citrate, 9 mM EDTA, pH 7.2) and was kept at 4°C until used.

Phenoloxidase activity (PO) was determined with spectrophotometry (DO 490 nm) through the formation of dopachrome pigment after the oxidation of the substrate l- 3,4-dihydroxyphenylalanine (l-DOPA, Sigma Chemical Co., USA), using the methodology described by Söderhäll and Häll (1984), and performed in triplicate. Serum samples were diluted (1:7) in TBS-2 (10 mM Tris, 336 mM NaCl, 5 mM $CaCl_2$, 10 mM $MgCl_2$, pH 7.6), and 50 µL of this solution were preincubated with an equal volume of the enzyme trypsin (Sigma, 1 mg mL^{-1}) for 15 min at 20°C in a 96 microwell plate (flat bottomed). In control, trypsin and serum were replaced by TBS-2. After incubation, 50 µL of l-DOPA (3 mg mL^{-1}) were added to the wells, and dopachrome formation was monitored after 5 and 10 min. One unit of specific activity from PO was equivalent to the variation of 0.001 in the absorbance min^{-1} mg^{-1} protein.

Protein concentration in hemolymph was estimated by a method described by Bradford (1976), using bovine serum albumin (BSA) as the standard.

To determine the agglutinin/lectin titer, 50 µL of a TBS solution (50 mM Tris, 5 mM $MgCl_2$, 10 mM $CaCl_2$, 150 mMNaCl, pH 7.4) was initially deposited in all the reservoirs of a microplate ("U" shaped base). Then 50 µL of the previously diluted serum (12x) in TBS was added to the first reservoir, followed by a serial dilution in the subsequent reservoir. Finally, 50 µL of a 2% dog erythrocyte solution (in 0.15 M NaCl) was added to each reservoir and the mixture incubated for 2 h in a humid chamber at room temperature. In the controls, the serum from the shrimp was replaced by TBS. The serum binder titer was expressed as the reciprocal of the largest dilution still capable of presenting agglutination. All tests were performed in duplicate.

Thermal shock

After the three-week period, 21 shrimp from each treatment were transferred simultaneously from the tanks with salt water at 29.3 ± 0.38°C, to 60 L aquariums containing salt water between 12.5 – 13.0°C under constant aeration, for one hour. The water temperature of the aquariums was monitored every 15 minutes. After this period, the shrimp were transferred simultaneously back to tanks with salt water at 29.3 ± 0.38°C, and survival was monitored for 24 hours after thermal shock (hact). The salt water used during the entire thermal shock phase came from the same reservoir, presenting the same salinity at 33.3.

Statistical analysis

Homoscedasticity and normality of data were assessed using the Levene test and the Shapiro-Wilk test, respectively. For hemato-immunological parameters, analysis of variance (one way ANOVA) was used, followed by the Tukey test. Survival data were subjected to the Kaplan-Meier test. All statistical tests were evaluated with a significance level of 5% and were performed on Statistica 13 (StatSoft).

RESULTS

Digestibility of Aurantiochytrium sp.

In general, the apparent digestibility coefficient (ADC) of *Aurantiochytrium* sp. was high (Table 4). The ADC for long-chain polyunsaturated fatty acids was around 60%, and for the n-3 group it was around 57%. The digestibility of dry matter was around 69% and protein was around 75%.

Immunological analysis

The total hemocyte count (THC) and the serum protein concentration (CP) of the shrimp showed no significant difference ($p \geq 0.05$). The serum binder titer was significantly higher ($p < 0.05$) in shrimp fed with *Aurantiochytrium* sp. meal in the additions of 0.5% and 2% when compared with the additions of 0 and 1%. Phenoloxidase (PO) activity was significantly higher ($p < 0.05$) in the group of shrimp fed with meal from the microalgae *Aurantiochytrium* sp. in the 1% addition group compared to the 0%; 0.5% and 2% additions ($p \geq 0.05$) (Table 5).

Table 4. Apparent digestibility coefficient of nutrients in *Aurantiochytrium* sp. meal for *Litopenaeus vannamei*.

Nutrient[a]	Apparent digestibility coefficient (%)
Dry matter	69.4±9.7
Protein	74.9±34.3
Lipids	55.7±2.1
14:0 Myristic acid	56.2±2.9
16:0 Palmitic acid	52.2±2.3
18:0 Stearic acid	59.0±7.7
18:1 n-9 Oleic acid	61.0±10.9
18:2 n-6 Linoleic acid	60.8±10.7
18:3 n-3 Linolenic acid	60.5±10.1
20:4 n-6 Arachidonic	100.0±7.1
20:5 n-3 EPA	59.7±8.8
22:6 n-3 DHA	55.6±1.9
SFA[b]	56.4±3.4
MUFA	60.9±10.9
PUFA	58.5±6.8
LC-PUFA n-6	60.0±9.3
LC-PUFA n-3	57.0±4.2

[a] Values expressed as the average of three repetitions, followed by the standard deviation. [b] Fatty acid groups: SFA = saturated, MUFA = monosaturated, PUFA = polyunsaturated. Analysis conducted at the Aquaculture Species Nutrition Laboratory - LABNUTRI/UFSC.

Table 5. Hemato-immunological parameters from *Litopenaeus vannamei* fed for 21 days on diets containing different levels of addition (0; 0.5; 1 and 2%) of the meal from the microalgae *Aurantiochytrium* sp.

Treatment	THC (x10^6 cells mL^{-1})	Agglutination Title (log, x + 1)	PO (min^{-1} mg^{-1} protein)	Protein concentration (mg mL^{-1})
0	32.8±10.7^a	10±0^b	22±14.2^b	262.5±22.7^a
0.5%	33.6±8.7^a	11.33±0.6^a	30.9±22.7^{ab}	286.1±52.8^a
1%	41.7±2.9^a	11±0^{ab}	53.8±12,5^a	278.5±18.9^a
2%	39.6±6.6^a	11.33±0.6^a	32.8±5.9^{ab}	285.6±16.1^a

THC = total hemocyte count; PO = activity of the phenoloxidase enzyme. * Mean values with common superscript letter(s) do not differ significantly in the Tukey test ($p < 0.05$). Data are presented as means ± standard deviation.

Thermal shock

L. vannamei shrimp fed the diet containing 0.5% and 1% of the microalgae *Aurantiochytrium* sp. showed better survival rates (%) after being subjected to thermal shock at low temperature compared to animals that were not fed the diet containing microalgae meal supplementation. Treatment with supplementation of 2% of the microalgae showed no difference in relation to the control (Figure 1).

Zootechnical parameters

In the feed additive test there was no statistical difference in animal survival, which was approximately 94.7 ± 1.8% for extreme treatments (0% and 2% substitution), and 89.3 ± 0.9% for other treatments. There was no statistical difference between the weights of shrimp fed the diet containing *Aurantiochytrium* sp. and control, with an average weekly growth rate of 1.15 ± 0.06 g.

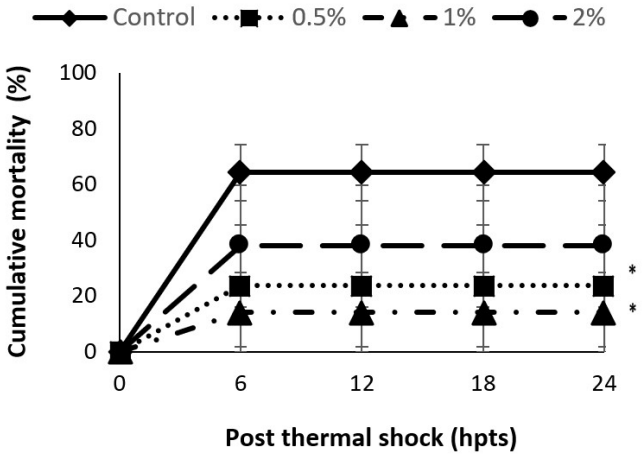

Figure 1. Cumulative mortality of *Litopenaeus vannamei* fed diets containing 0, 0.5, 1 and 2% of *Aurantiochytrium* sp., over 24 hours after thermal shock. There were significant differences (p <0.0001) between treatments 0.5% and 1% compared to the control. The error bars indicate the standard deviation of mean (n = 3).

DISCUSSION

The digestibility of *Aurantiochytrium* sp. meal was above 55%, with high utilization of protein and some of the fatty acids that are important and beneficial for the animals, such as arachidonic acid, linoleic acid, linolenic acid, eicosapentaenoic and docosapentaenoic. The digestibility of lipids and DHA fatty acid was also above 50%, demonstrating the utilization by the shrimp of the nutrients present in the algae meal.

Many studies demonstrate that the correct use of the dose/response ratio of immunostimulants improves the immune system of crustaceans, resulting in higher resistance to pathogens and stressors (Huang et al., 2006; Yangthong et al., 2016; Kitikiew et al., 2017). One example is the inclusion of algae which, due to its high nutritional value in terms of fatty acids and amino acids, improves the immune response, nutrient absorption and growth (Schleder et al., 2017; Guimarães et al., 2019).

Nonwachai et al. (2010) demonstrated a significant effect on the immune response of shrimp fed diets supplemented with seaweed rich in DHA and ARA (arachidonic) fatty acids. These authors suggest that the interaction between ARA and DHA may increase according to the unsaturation level of unsaturation, and that this is the first report on the beneficial effect of ARA on shrimp immunity.

In the present study, supplementation with the *Aurantiochytrium* sp. meal in the diet of *L. vannamei* did not affect the total hemocyte counts. However, the activity of the enzyme phenoloxidase (PO) was altered, being significantly higher for the shrimp fed with the inclusion of 1% of the microalgae meal. Schleder et al. (2017), added dry biomass of the brown macroalga *Undaria pinnatifida* to the feed with no resulting difference in the hemocyte count. However, at 4% inclusion, an increase in the activity of the phenoloxidase enzyme of *L. vannamei* was observed.

Regarding the protein concentration of hemolymph, there was no effect of the additon of the *Aurantiochytrium* meal in the diet. The serum binder titer was significantly higher (p <0.05) in the treatments with the addition of 0.5 and 2% of the microalgae meal. When crustaceans are subjected to conditions of stress or pathogen infection, there is a variation in the hemolymph binder titer (Barracco et al., 2014). The increase demonstrated may be related to an immune stimulating effect of these molecules.

In this research, feeding shrimp with the addition of 0.5% and 1% of the microalgae *Aurantiochytrium* sp. meal which has unsaturated fatty acids present, may have helped in the performance of the membrane fluidity of *L. vannamei* cells, and consequently, improved resistance to thermal stress, due to its high digestibility and utilization of nutrients. Similarly, Schleder et al.

(2017), through the analysis of MALDI-TOF MS, observed an increase in the fluidity of the membrane of shrimp hemocytes fed with 0.5% *Sargassum filipendula* after thermal shock with low temperature, demonstratingits important role inresistance to temperature variation.

Responses to thermal shock in aquatic animals vary according to the stability of the environment or the presence of different variables (Tomanek, 2008). This stress causes physiological impairments in ectothermic organisms such as failure of membrane integrity, protein interruption, oxidative stress, malfunction of mitochondrial breathing, loss of ionic homeostasis and neuromuscular coordination (Hayward et al., 2014). These impairments, mainly the reduction of membrane fluidity, impair cell function, affecting the transport of nutrients/ions, immobilizing membrane proteins, important mechanisms for resistance to cold (Pruitt, 1990; Hayward et al., 2014). The incorporation of saturated and unsaturated fatty acids in diets can improve the fluidity of the membrane, including for animals' intolerant to the cold, obtaining greater resistance (Teets and Denlinger, 2013; Hayward et al., 2014; Schumann, 2016).

Accordingly, the use of the microalgae *Aurantiochytrium* sp. evidence that the resistance that the shrimp acquires to the thermal shock is probably dependent on a multifactorial physiological response triggered by the algae supplementation. Thus, showing aviability for the shrimp farming in regions with unstable weather during crop season, like Southern Brazil.

CONCLUSION

Supplementation of 0.5% and 1% of *Aurantiochytrium* sp. in feeding *L. vannamei* grown in clear water results in an improvement in resistance to thermal shock. The digestibility of the *Aurantiochytrium* sp. meal and the utilization of nutrients by *L. vannamei* is high. Serum binder titer is incresed by the additions of 0.5 and 2% of the microalgae meal, and the phenoloxidase activity (PO) in the addition of 1%.

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REFERENCES

Alabi, A.O.; Jones, D.A.; Latchford, J.W. 1999. The efficacy of immersion as opposed to oral vaccination of *Penaeus indicus* larvae against *Vibrio harveyi*. Aquaculture, 178(1-2): 1-11. https://doi.org/**10.1016/s0044-8486(99)00131-3**.

AOAC – Association of Official Analytical Chemists. 1999. Official Methods of Analysis. 16th ed. Arlington: AOAC. 771p.

Barracco, M.A.; Perazzolo, L.M.; Rosa, R.D. 2014. Avances en la inmunología del camarón. In: Morales, V.; Cuellar-Angel, J. Guía técnica: patología e inmunología de camarones penaeidos. 2nd ed. Panamá: CYTED. p. 237-308.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2): 248-254. https://doi.org/**10.1016/0003-2697(76)90527-3**.

Chi, Z.; Liu, Y.; Frear, C.; Chen, S. 2009. Study of a two-stage growth of DHA-producing marine algae *Schizochytrium limacinum* SR21 with shifting dissolved oxygen level. Applied Microbiology and Biotechnology, 81(1): 1141-1148. https://doi.org/**10.1007/s00253-008-1740-7**.

Corrêa, C.F.; Nobrega, R.O.; Mattioni, B.; Fracalossi, D.M. 2018. Mixes of plant oils as fish oil substitutes for Nile tilapia at optimal and cold suboptimal temperature. Aquaculture, 497: 82-90. https://doi.org/**10.1016/j.aquaculture.2018.07.034**.

FAO – Food and Agricultural Organization of the United Nations. 2016. The state of world fisheries and aquaculture. Roma: SOFIA. 190p.

Folch, J.; Lees, M.; Sloane-Stanley, C.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226: 477-509.

Gong, H.; Lawrence, A.L.; Jiang, D.H.; Castille, F.L.; Gatlin, D.M. 2000. Lipid nutrition of juvenile *Litopenaeus vannamei*: I. Dietary cholesterol and de-oiled soy lecithin requirements and their interaction. Aquaculture, 190(3-4): 305-324. https://doi.org/**10.1016/S0044-8486(00)00414-2**.

Guimarães, A.M.; Schleder, D.D.; Nagata, M.; Nóbrega, R.O.; Fracalossi, D.M.; Seiffert, W.Q.; Vieira, F.N. 2019. *Aurantiochytrium* sp. meal can replace fish oil in practical diets for the juvenile Pacific white shrimp. Aquaculture Nutrition, 25(4): 798-807. https://doi.org/**10.1111/anu.12897**.

Harwood, J.L.; Guschina, I.A. 2009. The versatility of algae and their lipid metabolism. Biochimie, 91(6): 679-684. https://doi.org/**10.1016/j.biochi.2008.11.004**.

Hayward, S.A.L.; Manso, B.; Cossins, A.R. 2014. Molecular basis of chill resistance adaptations in poikilothermic animals. The Journal of Experimental Biology, 217(3): 6-15. https://doi.org/**10.1242/jeb.096537**.

Huang, X.; Zhou, H.; Zhang, H. 2006. The efect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and imune activy of the shrimp, *Fenneropeaneus chinensis*. Fish & Shellfish Immunology, 20(5): 750-757. https://doi.org/**10.1016/j.fsi.2005.09.008**.

Ju, Z.Y.; Deng, D.F.; Dominy, W. 2012. A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). Aquaculture, 354-355: 50-55. https://doi.org/**10.1016/j.aquaculture.2012.04.028**.

Kautsky, N.; Rönnbäck, P.; Tedengren, M.; Troell, M. 2000. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture, 191(1-3): 145-161. https://doi.org/**10.1016/S0044-8486(00)00424-5**.

Kitikiew, S.; Yu-Yuan, C.; Su-Tuen, Y.; Jiann-Chu, C. 2017. White Shrimp *Litopenaeus vannamei* that have received fucoidan show protective immunity after ammonia stressing. Taiwan Shuichanxue Hui Kan, 42(30): 189-197.

Li, Q.; Ai, Q.; Mai, K.; Xu, W.; Zheng, Y. 2013. A comparative study: In vitro effects of EPA and DHA on imune functions of head-kidney

macrophages isolated from large yellow croaker (*Larmichthys crocea*). Fish & Shellfish Immunology, 35(3): 933-940. https://doi.org/**10.1016 j.fsi.2013.07.004**.

Lightner, D.V. 2011. Virus diseases of farmed shrimp in the Western Hemisphere (the Americas): a review. Journal of Invertebrate Pathology, 106(1): 110-130. https://doi.org/**10.1016/j.jip.2010.09.012**.

Macias-Sancho, J.; Henrique Poersch, L.H.; Bauer, W.; Luis Alberto Romano, L.A.; Wasielesky, W.; Tesser, M.B. 2014. Fishmeal substitution with *Arthrospira* (*Spirulina platensis*) in a practical diet for *Litopenaeus vannamei*: effects on growth and immunological parameters. Aquaculture, 426-427: 120-125. https://doi.org/**10.1016/j.aquaculture.2014.01.028**.

Moser, J.; Álvarez, D.A.G.; Cano, F.M.; Garcia, T.E.; Molina, D.E.C.; Clark, G.P.; Marques, M.R.F.; Barajas, F.J.M.; López, J.H. 2012. Water temperature influences viral load and detection of White Spot Syndrome Virus (WSSV) i*n Litopenaeus vannamei* and wild crustaceans. Aquaculture, 326-329: 9-14. https://doi.org/**10.1016/j. aquaculture.2011.10.033**.

Neto, J.F.S.; Torres, V.M.; Lima, P.W.C.; Farias, W.R.L. 2008. Trial culture of marine shrimp *Litopenaeus vannamei* postlarvae under three feeding strategies. Ciência Agronômica, 39(3): 410-415.

Niu, J.; Chen, X.; Lu, X.; Jiang, S.; Lin, H.; Liu, Y.; Huang, Z.; Wang, J.; Wang Y., Tian, L. 2015. Effects of different levels of dietary wakame (*Undaria pinnatifida*) on growth, immunity and intestinal structure of juvenile *Penaeus monodon*. Aquaculture, 435: 78-85. https://doi. org/**10.1016/j.aquaculture.2014.08.013**.

Nonwachai, T.; Purivirojkul, W.; Limsuwan, C.; Chuchird, N.; Velasco, M.; Dhar, A.K. 2010. Growth, nonspecific immune characteristics, and survival upon challenge with *Vibrio harveyi* in Pacific white shrimp (*Litopenaeus vannamei*) raised on diets containing algal meal. Fish & Shellfish Immunology, 29(2): 298-304. https://doi.org/**10.1016/j.fsi.2010.04.009**.

NRC – National Research Council. 2011. Nutrient requirements of fish and shrimp. Washington: National Academic Press. 376p.

Pruitt, N.L. 1990. Adaptations to temperature in the cellular membranes of crustacea: membrane structure and metabolism. Journal of Thermal Biology, 15(1): 1-8. https://doi.org/**10.1016/0306-4565(90)90040-O**.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture, 172(1-2): 63-92. https://doi.org/**10.1016/S0044-8486(98)00436-0**.

Schleder, D.D.; Blank, M.; Peruch, L.G.B.; Vieira, F.N.; Andreatta, E.R.; Hayashi, L. 2017. Thermal resistance of Pacific white shrimp fed *Sargassum filipendula*: A MALDI-TOF mass spectrometry approach. Aquaculture, 481: 103-111. https://doi.org/**10.1016/j.aquaculture.2017.08.028**.

Schumann, J. 2016. It is all about fluidity: fatty acids and macrophage phagocytosis. European Journal of Pharmacology, 785: 18-23. https:// doi.org/**10.1016/j.ejphar.2015.04.057**.

Shah, M.R.; Lutzu, G.A.; Alam, A.; Sarker, P.; Chowdhury, M.K.; Parsaeimehr, A.; Liang, Y.; Daroch, M. 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology, 30(1): 197-213. https://doi.org/**10.1007/s10811-017-1234-z**.

Silva, J.W.A.; Santos, M.J.B.; Bezerra, J.H.C.; Damasceno, V.L.; Araujo, G.S.; Santos, E.S.; Moreira, R.T.; Lopes, D.N.M. 2020. Influência da microalga *Chlorella vulgaris* no desempenho zootécnico do camarão marinho *Litopenaeus vannamei*. Brazilian Journal of Development, 6(2): 5603-5614. https://doi.org/**10.34117/bjdv6n2-019**.

Söderhäll, K.; Häll, L. 1984. Lipopolysaccharide-induced activation of prophenoloxidade activating system in crayfish haemocyte lysate. Biochimica et Biophysica Acta, 797(1): 99-104. https://doi. org/**10.1016/0304-4165(84)90387-8**.

Strickland, J.D.H.; Parsons, T.R. 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin. Ottawa: Office des recherches sur les pêcheries du Canada. 310p.

Teets, N.M.; Denlinger, D.L. 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiological Entomology, 38(2): 105-116. https://doi.org/**10.1111/phen.12019**.

Teunissen, O.S.P.; Faber, R.; Booms, G.H.R.; Latscha, T.; Boon, J.H. 1998. Influence of vaccination on vibriosis resistance of the giant black tiger shrimp *Penaeus monodon* (Fabricius). Aquaculture, 164(1-4): 359-366. https://doi.org/**10.1016/S0044-8486(98)00200-2**.

Thanigaivel, S.; Chandrasekaran, N.; Mukherjee, A.; Thomas, J. 2016. Seaweeds as an alternative therapeutic source for aquatic disease management. Aquaculture, 464: 529-536. https://doi.org/**10.1016/j. aquaculture.2016.08.001**.

Tomanek, L. 2008. The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat shock response. Physiological and Biochemical Zoology, 81(6): 709-717. https://doi.org/**10.1086/590163**.

Van Wyk, P.; Scarpa, J. 1999. Water Quality and Management. In: Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K.L.; Mountain, J.; Scarpa, J. Farming marine shrimp in recirculating freshwater systems. Tallahassee: Florida Department of Agriculture & Consumer Services. pp. 141-162.

Wang, Y.; Li, M.; Filer, Y.; Xue, Q.; Mai, K. 2017. Replacement of fish oil with a DHA-rich *Schizochytrium* meal on growth performance, activities of digestive enzyme and fatty acid profile of Pacific white shrimp (*Litopenaeus vannamei*) larvae. Aquaculture Nutrition, 23(5): 1113-1120. https://doi.org/**10.1111/anu.12479**.

Willmer, P.; Stone, G.; Johnston, I. 2005. Environmental physiology of animals. 2nd ed. Malden: Blackwell Science, 754p.

Xia, S.; Zhao, P.; Chen, K.; Li, Y.; Liu, S.; Zhang, L.; Yang, H. 2012. Feeding preferences of the sea cucumber *Apostichopus japonicas* (Selenka) on various seaweed diets. Aquaculture, 344-349(1): 205-209. https:// doi.org/**10.1016/j.aquaculture.2012.03.022**.

Yangthong, M.; Hutadilok-Towatana, N.; Thawonsuwan, J.; Wutiporn, P. 2016. An aqueous extract from *Sargassum* sp. enhances the immune response and resistance against *Streptococcus iniae* in the Asian sea bass (*Lates calcarifer* Bloch). Journal of Applied Phycology, 28(6): 3587-3598. https://doi.org/**10.1007/s10811-016-0859-7**.

Yenari, M.A.; Giffard, R.G.; Sapolsky, R.M.; Steinberg, G.K. 1999. The neuroprotective potential of heat shock protein 70 (HSP70). Trends in Molecular Medicine, 5(12): 525-531. https://doi.org/**10.1016/ s1357-4310(99)01599-3**.

Zhou, Q.; Zeng, W.; Wang, T.; Wang, Y.; Xie, F. 2012. Dietary arginine requirement of juvenile Pacifc white shrimp, *Litopenaeus vannamei*. Aquaculture, 364-365: 252-255. https://doi.org/**10.1016/j.aquaculture.2012.08.020**.