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Haematococcus pluvialis biomass as a replacement for fish meal in the diet of *Macrobrachium amazonicum* post-larvae (Heller, 1862)

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ABSTRACT. The current study evaluates the effect of partial or total replacement of fish meal with *Haematococcus pluvialis* microalgae, cultivated in an NPK culture medium, on the growth performance of *Macrobrachion amazonicum* shrimp post-larvae. Four diets were formulated: control (without microalgae), 25, 50 and 100% *H. pluvialis* replacement. Only crude protein weight gain showed no interaction and was not selected for principal components analysis. The 25% fishmeal replacement in the diet was not effective, being similar to the control. However, the complete replacement of fishmeal with *H. pluvialis* promoted elevated survivability, length, weight and protein retention, making it the most relevant treatment for *M. amazonicum* post-larvae. Therefore, it is possible to completely replace fishmeal with the microalgae *H. pluvialis* in the diet of *M. amazonicum* shrimp, which improves growth performance.

Keywords: growth performance; microalgae; feed treatments; shrimp feed.

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Introduction

Several microalgae species are employed directly or indirectly as feed supplement for fish, mollusk and shrimp, at different growth stages. In time with appropriate size, high nutritional values, and growth rates, amino acids, antioxidants and disease resistance capacity, microalgae are being used successfully as aquaculture feed (Kumar, Sridhar, Jayappriyan, & Raja, 2023). Only a few strains of microalgae are used in the animal dietary due to the difference in culture techniques, toxicity, nutritional importance, shape, size and digestibility (Daniel et al., 2015). The green alga *Haematococcus pluvialis* presents a significant commercial value due to an elevated production of astaxanthin, an important natural antioxidant, with high amino acids, and protein contents, with high biologic value and as immune-stimulating agent (Zhao et al., 2023). *Haematococcus pluvialis*, cultured in an inorganic fertilizer medium (NPK 10:10:10), featured high cell density, with a great profile of amino acids levels and improved algal development, when compared to commercial medium WC (Scardoeli-Truzzi & Sipaúba-Tavares, 2017).

Depending on the algal species and their growth conditions, they may contain up to 60% protein, 60% carbohydrate, or 70% oils, and produce valuable pigments, growth promoting substances, hormones and secondary metabolites that provide natural antioxidant, antimicrobial, anti-inflammatory and anti-immuno-stimulants beneficial to aquatic animals (Yarnold, Karan, Oey, & Hankamer, 2019). Antioxidant ingredients, naturally available in carotenoids, are important for the coloration of muscle and skin of aquatic animals (Martinez-Delgado et al., 2020).

Several species of microalgae have been tested to compose shrimp feed and was revealed that many species had enhancing growth effects (Ju, Deng, & Dominy, 2012). Shrimps are omnivorous organisms and microalgae compose their natural diet. Moreover, *H. pluvialis* meal has never been investigated as a protein source in post-larvae of native shrimp such as *Macrobrachium amazonicum*.

Macrobrachium amazonicum shrimp occurs in the Amazon River, floodplains, lakes, and reservoirs in the tropical and subtropical regions of South America. The distribution of the species includes the Amazon, Orinoco, São Francisco, and Prata river basins, and the rivers of north-eastern and midwestern Brazil. Larvae are planktonic and feed on zooplankton and inert particles, whereas juveniles and adults show epibenthic habits and feed on detritus, algae, macro- and micro-invertebrates, and fish (Maciel & Valenti, 2009).

Similar to other animals, shrimps are unable to biosynthesize carotenoids, which are obtained through the microalgae consumption. Shrimp aquaculture has increased and is currently facing increased costs due to feed ingredients. Consequently, research on the identification of suitable alternatives without jeopardizing crustacean production is underscored. The inclusion of dietary microalgae in the diets of the Pacific white shrimp has proved to be highly promising, mainly by complete replacement of fish oil with different combinations of microalgae oil, soybean oil and flax oil in plant protein-based diets resulting in growth and survival similar commercial diets (Allen, Thompson, Filer, Tidwell, & Kumar, 2019). A key advantage in focusing on microalgae as an ingredient is due to their higher nutrient profile including omega-3, fatty acids and essential amino acids (Idenyi, Eya, Nwankwegu, & Nwoba, 2022).

Fish meal has been used as the major source of protein in aquafeeds. Since fish meal and other main ingredients used in aquaculture are limited, feed formulations require replacement ingredients, such as microalgae. The latter are commercially accessible on the market and may be introduced in artificial feed (Daniel et al., 2015). The need for alternative protein source to fishmeal has highlighted many biomass products that are local or regional in nature such as microalgae (Radhakrishnan, Belal, Seenivasan, & Muralisankar, 2016).

Haematococcus pluvialis microalgae cultured in NPK culture medium demonstrated that protein and lipid contents were enough to maintain the biomass content around 1 g L^{-1} and amino acids concentrations above 1.2 g 100 g^{-1}, except methionine and histidine. Thus, it may be emphasized that the alternative medium based on inorganic fertilizer in the proportion of 10:10:10 may be a viable tool to cultivate *H. pluvialis* with lower production cost when compared to the commercial WC (Wright Chu) medium (Scardoeli-Truzzi & Sipaúba-Tavares, 2015).

Microalgae may be a part of the major feed ingredients, but its supplementation in artificial diet requires large proportions of biomass. A key challenge is to develop aquafeeds that may provide high nutritional quality, maintain sustainable food systems and ensure that yields meet increasing demand. Investigating alternative protein sources to be added to the feed is crucial. Current study evaluated the effect of partial or total replacement of fish meal by *H. pluvialis* microalga, cultured in NPK culture medium, on post-larvae growth performance of *Macrobrachium amazonicum* shrimp. This study analyses whether: (1) *H. pluvialis* microalgae may be employed as a replacement of the fishmeal; (2) which percentage (25, 50, and 100%) of microalgae may be managed as a feed diet to post-larvae of *M. amazonicum* shrimp; (3) growth performance was influenced by different feed diet treatments (control, 25, 50, and 100%).

Material and methods

Microalgae strain and culture conditions

Haematococcus pluvialis (CMEA 227 C1) was cultured in laboratory conditions in triplicate, using inorganic fertilizer medium (NPK 10:10:10) (Scardoeli-Truzzi & Sipaúba-Tavares, 2017) in a batch-cultured system at 22±1°C, with a continuous light intensity of about 30 µmol photon m^{-1} s^{-1}. Approximately 50 g L^{-1} of inorganic fertilizer were added to 1 L water and autoclaved at 1 atm during, 30 minutes. The experiment started with a 250 mL flask at a microalgae density of 1 x 10^5cells mL^{-1} cultured in WC medium. When culture reached the late exponential growth rate phase (14 days), 250 mL of microalgae were placed in a 2L Erlenmeyer, adding approximately 280 mL of NPK medium. The volume was completed with sterilized distilled water. The experiment was performed in 2 L volumes with continuous air bubbling. Vitamin B_{12} complex was added to NPK medium at a rate of 0.02 g L^{-1}, plus biotin (0.01 mg L^{-1}) (Sipaúba-Tavares, Pelicioni, & Olivera, 1999). *Haematococcus pluvialis* microalgae were cultivated for 28 days for algal biomass, with three replicates.

Experimental design and growth performance

Assays were performed with *M. amazonicum* post-larvae (Heller, 1862), acquired from the Crustacean Sector of the Aquaculture Center from the Sao Paulo State University (UNESP) (21°15'19"S and 48°19'21"W), Brazil. The post-larvae used in this study were 5 days post-metamorphosis. The feeding trial was conducted indoors in polypropylene tanks with individual water recirculation system, constant aeration and thermostat with an effective volume of 60 L. The experimental design was randomly divided into 16 experimental tanks (four treatments and four replicates) with light/dark cycles at a 12h:12h cycle and 120 shrimp post-larvae stocked in each experimental unit. The experiment was carried out for 30 days. Initial weight of shrimp in all treatments was 0.01±0.003 mg, with initial length at 11.8±1 mm. Mean post-larvae shrimp weight at the start

and end of the experiment was determined. The post-larvae of each dietary treatment were weighted individually on digital balance (±0.1 µg Mettler Toledo) and their length gain was determined with a digital vernier caliper (± 0.001 mm Mitutoyo, model CD-6 CS). Weight gain and total length of post-larvae shrimp were measured one by one. Post-larvae specimens were fed twice a day (9:30 a.m. and 17:30 p.m.) with a feed rate of 40% of biomass following Araújo and Valenti (2005).

The following formulae were used to assess growth performance:

specific growth rate (SGR%) = [ln (final weight) - ln (initial weight)/number of days] x 100

survival rate (%) = [number of surviving shrimp / total number of stocked shrimps] x 100 weight gain (mg) = final mean shrimp weight - initial mean shrimp weight

feed conversion ratio = feed intake / weight gain

length gain (mm) = final mean shrimp length - initial mean shrimp length

food consumption (mg ind^{-1}) = mean fed consumption/final number of individuals

protein efficiency ratio = mean weight gain / crude protein intake

protein retention efficiency (%) = [(Cpf x Wf) - (Cpi x Wi)/crude protein intake x100]

crude protein on weight gain (%) = [(Cpf x Wf) - (Cpi x Wi)/Wf – Wi x 100]

Feed treatments

Four diets were formulated at different levels of *H. pluvialis* substituting fish meal including 0% (control, without microalgae), 25, 50 and 100%. The control was formulated to contain 35% crude protein, featuring 3,936 kcal kg^{-1} gross energy. Tested ingredients were ground in a hammer mill, mixed, moistened and pelleted in a meat grinder. The pellets were dried for 24 hours at 50°C, fractionated and sieved to obtain a diameter of 0.05 mm. Table 1 shows ingredients and proximate composition of the experimental diets.

Table 1. Ingredients, formulation and proximate composition of experimental diet with 0% (control), 25, 50, and 100% substitution of fishmeal by *Haematococcus pluvialis* meal.

Ingredients (g kg^{-1})	Microalgae Treatments			
	Control	25%	50%	100%
Microalgae[a]	0	5	10	20
Fish meal[b]	20	15	10	0
Soybean meal[b]	30	28	27.5	30
Meat and bone meal	6.4	6.4	7.5	10
Corn[b]	15.8	13.8	10.5	0.23
Wheat flour[b]	16.8	16.8	16.8	16.8
Poultry by-product meal[b]	5	8.5	10.2	11.5
Rice bran[b]	1	1	1	1
Cellulose	1.1	1.2	1.5	1.8
Lysine	0.4	0.4	0.4	0.6
Methionine	0.2	0.25	0.25	0.5
Threonine	0.5	0.5	0.5	0.8
Soybean oil[b]	1.7	1.7	1.7	2.6
Dicalcium phosphate	0	0.3	1	3
Salt	0.5	0.5	0.5	0.5
Mineral-vitamin mix[c]	0.5	0.5	0.5	0.5
Antifungal (Phylax®)	0.15	0.15	0.15	0.15
BHT[d]	0.02	0.02	0.02	0.02
Proximate composition[e]				
Dry matter (%)	94.09	94.01	93.44	94.23
Crude protein (%)	35.03	35.45	34.82	35.71
Ether extract (%)	6.67	7.35	7.08	7.39
Total ash (%)	11.97	11.85	11.39	10.49
Gross energy (kcal kg^{-1})	3,936.06	3,955.77	3,880.41	3,971.39

a Microalgae obtained locally. Biochemical composition of Haematococcus pluvialis: 34.1% protein; 4.1% lipid; b Fish meal, soybean meal, corn, wheat flour, poultry by-product meal, rice bran, soybean oil obtained locally. The proximate composition of ingredients were fish meal: protein = 541.4; ether extract = 105.7; crude energy (kcal kg^{-1}) = 4,074; soybean meal: protein = 452.7; ether extract = 18.4; nitrogen-free extract = 292.6; crude energy (kcal kg^{-1}) = 4,167; corn: protein = 77.9; ether extract = 48.1; nitrogen-free extract = 726.3; crude energy (kcal kg^{-1}) = 4,003; wheat flour: protein = 157.0; ether extract = 40.4; nitrogen-free extract = 571.0; crude energy (kcal kg^{-1}) = 4,093; poultry by product meal: protein = 635.1; ether extract = 137.9; crude energy (kcal kg^{-1}) = 4,834; rice bran: protein = 116.8; ether extract = 157.2; nitrogen-free extract = 421.2; crude energy (kcal kg^{-1}) = 4,471; soybean oil: ether extract = 1000.0; crude energy (kcal kg^{-1}) = 9,329; c Each 1% contains: folic acid (1 mg); pantothenic acid (20 mg); antioxidant (125 mg); choline (150 mg); copper (10 mg); iron (100 mg); iodine (5 mg); manganese (70 mg); selenium (0.15 mg); vitamin A (3,000 IU kg^{-1}); vitamin B (16 mg); vitamin B12 (20 mg); vitamin B2 (8 mg); vitamin B6 (3 mg); vitamin C (350 mg); vitamin D3 (3000 IU kg^{-1}); vitamin E (200 IU kg^{-1}); vitamin K (6 mg); zinc (150 mg); niacin (100 mg); biotin (0.10 mg); d BHT: butyl-hydroxytoluene or butylated hydroxytoluene, is a lipophilic organic compound with antioxidant properties; e Chemical composition was calculated by analyzing all ingredients prior to use in experimental diets, and the data obtained were used as basis for feed formulation (crude protein; % ash; % dry matter and % ether extract) (AOAC, 2012). Gross energy: calculated by oxygen bomb colorimeter.

Analytical methods

The amino acids profile of *H. pluvialis* was cultured in NPK (10:10:10) according Scardoeli-Truzzi and Sipaúba-Tavares (2017). The total length of 50 specimens was determined with microscope Leica DFC 295 by image analysis system LAS Core (LAS V3.8), with a 400X micrometric objective. Cell volume was calculated by mean cell size with the most appropriate geometric form, or rather, the sphere formula (Hillebrand, Dürselen, Kirschtel, Pollingher, & Zohary, 1999). Total organic carbon was calculated with formula $C = 0.1204V^{1.051}$ (C = carbon content in pg cel^{-1}, V = cell volume) (Rocha & Duncan, 1985). Chlorophyll-*a* concentration was determined by extracting pigments with alcohol 90% and reading spectrophotometer (663 and 750 nm) processed according to methodology by Nusch (1980). Microalgae biomass was harvested, centrifuged, and lyophilized for analysis of total lipids content (Association of Official Analytical Chemists [AOAC], 2012), and protein content by Dumas combustion method, supplied by Leco (CN628), whilst gross energy was measured by oxygen bomb calorimeter.

The shrimp water parameters of each experimental tank were performed weekly at 8:00h, for 30 days. Water dissolved oxygen, conductivity and pH were measured in situ with a multi-sensor YSI 556 MPS. Total inorganic nitrogen and total phosphorus were quantified by spectrophotometer, following Koroleff (1976) and Golterman, Clymo, and Ohnstad (1978). Only water temperature was monitored daily. The analysis contents of diet treatments were dry matter, total ash, crude protein, ether extract and gross energy (AOAC, 2012).

Statistical analysis

The normality of data distribution was assessed by Kolmogorov–Smirnov test. Rates differences of water variables for each treatment by fed diet with 0% (control), 25, 50, and 100% replacement of *Haematococcus pluvialis* were analysed by one-way analysis of variance (ANOVA), followed by Duncan's post-hoc analysis for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. All experiments were carried out in triplicate for microalgae and in four replicates for post-larvae shrimp. Growth performance data were evaluated by Principal Components Analysis (PCA) and undertaken according to software Statistic 10 (StatSoft Inc., 2007).

Results and discussion

After a 28 days cultivation period of *H. pluvialis*, approximately 1 g L^{-1} of dry biomass with high gross energy (3,991 kcal kg^{-1}) was obtained. Protein content was 34% biomass dry weight, although lipids content was lower, about 4% biomass dry weight. Chlorophyll-*a* concentration (0.74 ± 0.5 mg L^{-1}) and total organic carbon (1,148 ± 541 pg $cell^{-1}$) were high, and total length and cell volume were 22 µm and 6,051 $µm^3$, respectively (Table 2). Amino-acids were above 1.2%, except methionine and histidine (0.6%). However, leucine (3.4%) and glutamic acid (3.2%) had the highest amino-acids contents in *H. pluvialis* biomass. Other amino-acids rates were between 1.2% (tyrosine) and 2.9% (arginine) (Table 2).

Table 2. Amino acids profile and biological data of *Haematococcus pluvialis* microalgae cultured in inorganic fertilizer (NPK, 10:10:10) medium.

Amino Acids	g $100g^{-1}$ dry biomass
Alanine	2.7 ± 0.02
Arginine	2.9 ± 0.01
Glutamic acid	3.2 ± 0.02
Histidine	0.6 ± 0.01
Leucine	3.4 ± 0.06
Lysine	2.1 ± 0.04
Methionine	0.6 ± 0.00
Proline	1.8 ± 0.04
Serine	1.5 ± 0.00
Threonine	1.6 ± 0.01
Tyrosine	1.2 ± 0.12
Valine	2.1 ± 0.13
Biological Data	
Chlorophyll-*a* (mg L^{-1})	0.74 ± 0.5
Total organic carbon (pg $cell^{-1}$)	1,148 ± 541
Total length (µm)	22 ± 3
Cell volume ($µm^3$)	6,051 ± 2,685
Protein (% DW)*	31.6 ± 5
Lipids (% DW)*	6 ± 2

*DW = dry weight.

Microalgae-based feeds offer promising food source and its bioactive compounds in microalgae biomass may enhance the post-larvae shrimp growth performance due to micronutrients and amino-acids in the microalgae biomass.

Relative concentration of dry matter in the control diet and in other dietary treatments was above 93%. Crude protein and gross energy were similar across all levels of microalgae supplementation, although the ether extract content was higher (above 7%) in shrimp diet with different levels of *H. pluvialis* (Table 1). Growth performance and survival rate of post-larvae *M. amazonicum* diet containing different levels of *H. pluvialis* are shown in Table 3.

Table 3. *Macrobrachium amazonicum* growth performance with feed diet featuring 0% (control), 25, 50, and 100% substitution of *Haematococcus pluvialis* meal.

Growth Parameters	Microalgae Treatments			
	Control	25%	50%	100%
Weight gain (mg)	42.1 ± 6	43.4 ± 9	47.1 ± 8	52.8 ± 3
Length gain (mm)	7.5 ± 0.9	8.0 ± 1.4	8.6 ± 0.8	9.9 ± 0.6
Specific growth rate (%)	5.4 ± 0.4	5.4 ± 0.5	5.7 ± 0.5	6.0 ± 0.2
Feed conversion ratio (g g^{-1})	4.0 ± 0.8	3.9 ± 0.9	3.4 ± 0.6	2.7 ± 0.2
Food consumption (mg ind^{-1})	167 ± 17.7	165 ± 17.2	160 ± 9.9	144 ± 14
Protein efficiency ratio	2.4 ± 0.3	2.4 ± 0.5	2.7 ± 0.5	3.0 ± 0.2
Protein retention efficiency (%)	53 ± 9	56 ± 5	61 ± 10	68 ± 3
Crude protein on weight gain (%)	11.9 ± 0.7	12.3 ± 0.5	11.8 ± 1.2	11.9 ± 0.3
Survival rate (%)	75.4 ± 7	76.5 ± 8	78.3 ± 5	87.1 ± 8

Although protein productive values and length gain were higher in post-larvae shrimp feed containing 50 and 100% substitution of microalgae, control and 25% levels of microalgae supplementation were below 56%. The opposite was observed to feed conversion ration where the lower content was obtained in shrimp feed containing 50 and 100% substitution of microalgae at an average of 3.4 and 2.7, respectively (Table 3).

The use of natural products to improve the yield of laboratory-bred organisms have increased since they respond positively with high survival rates and contribute to the increase in protein assimilation and feed utilization. Ju, Deng, and Dominy (2012) observed that *Peneaus vannamei* fed on *H. pluvilais*, had a high survival (between 87.5 and 100% diet microalgae biomass) and growth rate (1.25 g wk^{-1}), with up to 50% replacement of the fish meal. The study showed that survival rate was 87.1% when shrimp was fed on a diet containing 100% substitution with microalgae. However, growth performance such as crude protein was similar in the control (without microalgae), 50 and 100% levels of microalgae supplementation.

Hassan and Chakrabarti (2009) stated that only about 10-15% of dietary protein requirement may be met by algae protein. Shapawi, Basri, and Shaleh, (2017) reported that when *Penaeus vannamei* juveniles were fed with 10% of *H. pluvialis* replacing the fish meal protein, the growth rate was high (1.5% day^{-1}) when compared to that in other levels (20, 30 and 40%). However, the opposite has been reported in the current study with 100% substitution of microalgae that improved growth performance and survival rate, such as increased weight, length gain and protein. Xu, Pan, Zao, and Huang, (2012) reported that protein retention efficiency improved due to two main factors: (1) the ability to better digest the proteins made available in the food and (2) the nutritional benefits provided as a supplemental and essential protein source. Feeding trials conducted on shrimp revealed that the microalgae *H. pluvialis* had a positive impact on growth and resulted in favorable survival rates. These trials also confirmed that the incorporation of the microalgae into shrimp feed is a valuable ingredient as food source.

According to Xie et al. (2018) the effect of supplementing *H. pluvialis* on growth performance of shrimp primarily depends on differential astaxanthin requirement and the various sources of *H. pluvialis*. However, these authors also suggest that *H. pluvialis* could enhance the resistance of shrimp to environment stress and improve survival rates. It remains unclear which factors or compounds contained in the microalgae contribute to the enhancement of shrimp growth. Wade et al. (2015) found that the weight of juvenile black tiger shrimp was significantly higher when *H. pluvialis* was included in the diet compared to the control diet (0% microalgae). According to Diaz-Jiménez, Hernandez-Vergara, Pérez-Rostro, and Ortega-Clement (2019), crustaceans select different sources of microalgae based on their food preferences and metabolic requirements. Omnivorous crustaceans may adjust their metabolic activity according to the availability or the quality of the feed.

Parisenti et al. (2011) reported that the use of *H. pluvialis* microalgae is beneficial to shrimp development, weight gain and survival rates because of astaxanthin. The latter provides antioxidant protection, which increases resistance to physiological and environment stress, while enhancing survival rate. Diaz-Jiménez et al. (2019) demonstrated that microalgae astaxanthin improved survival rate when compared to that in control group (without astaxanthin) due to carotenoids that eliminate free radicals which positively impact on shrimp health state and survival. The feeding of aquatic animals with required nutrients plays a crucial role, whilst carotenoids such as astaxanthin, are included in the feed in various forms to obtain skin color and other benefits (Weeratunge & Perera, 2016).

Principal components analysis (PCA) of biological index retained 99.83% of the original data variables in the first two axis (Factor 1 = 99.01%; Factor 2 = 0.82%). Only crude protein weight gain showed no interaction and was not selected for PCA. Control and T25% were grouped on the positive side axis of principal component 1, along with FCR (feed conversion ratio) and FC (food consumption). The replacement of 25% of fishmeal with *H. pluvialis* in the diet was ineffective, as it was similar the control (without microalgae). The interaction of FCR and FC with T25% and the control may be associated with higher feed consumption with lower level of microalgae replacement in the feed diet. The replacement of 50% of fishmeal (T50%) with *H. pluvialis* in the feed diet showed an inverse proportion to the control and T25%. The negative side of the principal component indicated an association with 100% fishmeal replacement by *H. pluvialis* in the feed diet, which promoted high survival rates, increased length, weight and protein content. This level was the most significant treatment for post-larvae of *M. amazonicum* (Figure 1).

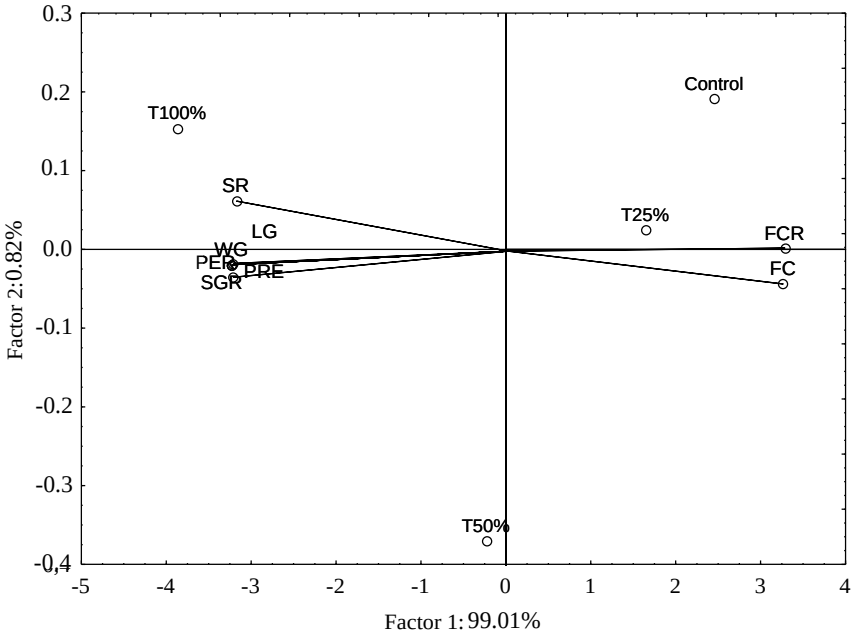

Figure 1. Results of the principal component analysis (PCA) for biological index of *Macrobrachium amazonicum* where: Control, T100, T50 and T25% = levels of *Haematococcus pluvilais* in feed treatments; SGR = specific growth rate; SR = survival rate; WG = weight gain; LG = length gain; FCR = feed conversion ratio; FC = food consumption; PER = protein efficiency ratio; PRE = protein retention efficiency.

Controlled conditions in the laboratory were appropriate for the shrimp diet experiment, since the results of water quality variables were similar ($p > 0.05$) across the different levels of microalgae supplementation. The pH remained alkaline, dissolved oxygen levels were above 7.1 mg L^{-1}, and conductivity exceeded 300 µS cm^{-1} (Table 4). Total inorganic nitrogen showed no significant differences with *H. pluvialis* supplementation. Total phosphorous was three times higher than total inorganic nitrogen, with the highest concentration achieved with a feed diet at 100% replacement (241 µg L^{-1}) (Table 4). Water quality, including the alkaline pH and high total phosphorous in all treatments, did not interfere with *M. amazonicum* growth. The increase in the microalgae increment in the feed led to a rise in the amount of total phosphorus dissolved in the culture water.

Using *Haematococcus pluvialis* for partial and total replacement of fish meal had no adverse effects on the growth performance of post-larvae shrimp. Microalgae may have contained the necessary components for growth in sufficient proportions to meet the minimum nutritional requirements for shrimp growth. The use

of microalgae biomass in aquaculture technology is highly relevant and plays a central role in aquaculture industry. However, further studies should be conducted due to the numerous microalgae species found in both fresh and marine water. Additionally, the limited studies conducted so far have already indicated that microalgae are significant feed ingredients in aquaculture.

Table 4. Water variables of each tank of *Macrobrachium amazonicum* with feed diet featuring 0% (control), 25, 50, and 100% substitution of *Haematococcus pluvialis* meal. Data are expressed as means ± standard deviation. Values in the same row with different superscript are significantly different (p < 0 .05).

Water Variables	Microalgae Treatments			
	Control	25%	50%	100%
Dissolved oxygen (mg L^{-1})	7.2 ± 0.02[a]	7.2 ± 0.05[a]	7.2 ± 0.1[a]	7.1 ± 0.1[a]
Conductivity (µS cm^{-1})	306 ± 15[a]	305 ± 13[a]	309 ± 10[a]	301 ± 27[a]
pH	8.4 ± 0.06[a]	8.5 ± 0.01[a]	8.4 ± 0.04[a]	8.3 ± 0.2[a]
Total inorganic nitrogen (µg L^{-1})	26.6 ± 15.2[a]	41.7 ± 21.2[a]	50.4 ± 9.4[a]	48.3 ± 25.9[a]
Total phosphorous (µg L^{-1})	124 ± 35.6[c]	112 ± 15.1[c]	184 ± 22.2[b]	241 ± 20.7[a]

Conclusion

Diets containing 50 and 100% *Haematococcus pluvialis* yielded positive outcomes. Post-larvae shrimp fed with a 100% microalgae-substituted diet exhibited robust protein productivity, a favorable protein efficiency ratio, increased length and weight gains, higher specific growth rates, and improved survival rates. In diets with 50 and 100% microalgae replacing animal meal, weight gain increased by 10.7 and 20.1%, respectively. Completely substituting fishmeal with *H. pluvialis* in post-larvae *M. amazonicum* diets enhanced growth performance and survival. *H. pluvialis* holds significant potential as a valuable early-stage food source for native shrimp development.

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