



# Aquaculture effluent-based cultivation of *Scenedesmus acuminatus* and its application as an ingredient in tambaqui (*Colossoma macropomum*) diets

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## Abstract

Aquaculture generates large volumes of nutrient-rich effluents that, if untreated, contribute to eutrophication. Microalgae-based phycoremediation is a sustainable strategy for recycling these effluents while producing high-nutritional-value biomass. This study evaluated the production performance, nutritional composition, and fatty acid profile of *Scenedesmus acuminatus* cultivated in aquaculture effluent and its inclusion in diets for juvenile tambaqui (*Colossoma macropomum*). Effluent from intensive *Brycon amazonicus* tanks was used as the sole nutrient source for cultivation in photobioreactors. The resulting biomass showed consistent production, high crude protein content (52.1%), relevant vitamin E levels (49.04 mg kg<sup>-1</sup>), and the presence of polyunsaturated fatty acids, including omega-3. The biomass was incorporated into five isonitrogenous (32.2% crude protein) and isoenergetic (17.22 MJ kg<sup>-1</sup>) diets: a control (SA0%) and four inclusion levels (SA1%, SA2%, SA3%, and SA5%). Juvenile tambaqui were fed the experimental diets for five weeks, and growth performance, hematological parameters, whole-body and fillet proximate composition, and fatty acid profiles were evaluated. Across all inclusion levels (1–5%), no significant differences were observed in survival, feed intake, growth performance, or whole-body and fillet proximate composition. Higher inclusion levels (SA3% and SA5%) were associated with increased thrombocyte and neutrophil counts, indicating immunomodulatory responses. The SA3% treatment resulted in a 45% higher omega-3 content in the fillet than the control; however, no statistical differences were detected among treatments ( $p=0.21$ ), and the 5% inclusion level did not yield additional increases. These findings reinforce the feasibility of producing *S. acuminatus* biomass from aquaculture effluent and its use as a functional dietary ingredient, supporting circular bioeconomy strategies for sustainable aquaculture.

**Keywords** Circular economy · Microalgae-based feed · Functional aquafeed · Sustainable aquaculture · Omega-3 enrichment

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## Introduction

Projections indicate that the global population may reach 9.8 billion by 2050, intensifying demand for food production, particularly for high-biological-value proteins (UN 2019). In this context, aquaculture has consolidated its role as a strategic sector capable of meeting growing food demands while contributing to global food security (FAO 2022; Pennells et al. 2025). Currently, aquaculture accounts for approximately 51% of the 185 million tons of aquatic animals produced worldwide (FAO 2024). Sustaining this growth, however, requires overcoming significant constraints, including limited space for expansion, increasing competition for traditional feed ingredients such as soybean meal, and the need for more efficient water use (Yadav et al. 2024).

To address these challenges, production systems have progressively shifted toward models that prioritize efficiency and sustainability. Recirculating aquaculture systems (RAS), for example, operate as closed or semi-closed systems in which water is continuously treated and reused through mechanical and biological filtration (Gupta et al. 2024). In contrast, biofloc technology (BFT) relies on the formation of microbial aggregates that assimilate nitrogenous wastes and convert them into microbial biomass consumed by cultured organisms (Lovejan et al. 2026). While RAS emphasizes water reuse and treatment efficiency, BFT promotes internal nutrient recycling. Despite these advances, high stocking densities inherent to such systems generate considerable loads of organic and inorganic residues, particularly nitrogen and phosphorus derived from feces, uneaten feed, and fertilizers (Magnotti et al. 2016; Huang et al. 2024). Consequently, effluent treatment or reuse becomes essential, as direct discharge often fails to meet environmental standards.

In this scenario, Integrated Multi-Trophic Aquaculture (IMTA) has emerged as a promising strategy to mitigate environmental impacts while improving resource-use efficiency (Beal et al. 2018). By integrating species at different trophic levels, IMTA promotes nutrient cycling and can enhance both productivity and profitability (Nissar et al. 2023; Costa et al. 2025). Among the various integration approaches, coupling fish farming with microalgae cultivation has received increasing attention. Microalgae have demonstrated high efficiency in removing nutrients from aquaculture effluents, with reported removal rates of up to 98% for nitrogen and 97% for phosphorus, and they also contribute to CO<sub>2</sub> sequestration (Alazaiza et al. 2022; de Cassia Soares Brandão et al. 2023). Beyond their bioremediation capacity, microalgal biomass is rich in proteins, pigments, vitamins, and polyunsaturated fatty acids, making it an attractive ingredient for feed formulation (Yarnold et al. 2019; Osathanunkul et al. 2025).

The incorporation of microalgae into aquafeeds has been widely investigated as a means to partially replace fishmeal or fish oil, to supply alternative protein sources, and to enhance growth performance, immune responses, and fillet nutritional quality. Frequently studied genera include *Arthrospira* (Spirulina), *Chlorella*, *Scenedesmus*, *Nannochloropsis*, and *Tetraselmis*, which have been evaluated in species such as Atlantic salmon (*Salmo salar*), gilthead seabream (*Sparus aurata*), Nile tilapia (*Oreochromis niloticus*), and white shrimp (*Litopenaeus vannamei*) (Sørensen et al. 2017; Maryshamya et al. 2019; Pereira et al. 2020; Sharawy et al. 2020; Silva et al. 2023; Ferreira et al. 2024). In many cases, improvements have been reported in growth, pigmentation, antioxidant capacity, and fatty acid enrichment. However, most of these studies rely on biomass cultivated in standardized commercial media (Gong et al. 2019; Ferreira et al. 2024). Comparatively little attention has been given to the nutritional and functional properties of microalgae produced directly in fish farming effluents, even though cultivation conditions are known to significantly

influence the biochemical profile of microalgae (Sipaúba-Tavares et al. 2017; Bhatti et al. 2023).

*Scenedesmus acuminatus* is a robust freshwater species characterized by high protein content and relevant levels of polyunsaturated fatty acids. Its ability to grow efficiently in nutrient-rich effluents makes it a suitable candidate for integration into aquaculture systems that aim to valorize waste and produce biomass. *Colossoma macropomum* (tambaqui), an omnivorous Amazonian species of major commercial importance, was selected as the experimental model due to its regional relevance and dietary plasticity, which allows for the practical evaluation of alternative feed ingredients under farming conditions.

Therefore, the present study aimed to characterize the production and nutritional composition of *S. acuminatus* biomass cultivated in fish-farming effluent and to evaluate the effects of including it in the diet of juvenile *C. macropomum* on growth performance, health parameters, and the nutritional composition of the fish fillet.

## Material and methods

### Study design

#### Production of biomass using *S. acuminatus*

Effluent from a tank containing *Brycon amazonicus* ( $2.28 \text{ kg m}^{-3}$ ) was first collected and used for microalgal biomass production. The effluent composition included nitrate ( $182.32 \text{ mg L}^{-1}$ ), ammonia ( $1.75 \text{ mg L}^{-1}$ ), total suspended solids ( $278.48 \text{ mg L}^{-1}$ ), orthophosphate ( $0.35 \text{ mg L}^{-1}$ ), alkalinity ( $83 \text{ mg L}^{-1} \text{ CaCO}_3$ ), and pH (7.37). The microalgae were subsequently cultivated in four vertical tubular photobioreactors, each with a working volume of 200 L. The effluent was pumped into a sedimentation tank using a submersible pump and underwent a filtration process. Initially, it was filtered through polyethylene meshes of 55  $\mu\text{m}$  and 20  $\mu\text{m}$ . Subsequently, it passed through a gravel and sand filter composed of pebbles of different granulometry. After filtration, the water was stored in a  $2 \text{ m}^3$  tank, where  $20 \text{ mg L}^{-1}$  of NaClO was added, followed by continuous aeration for 24 h for disinfection (Castro-Mendes et al. 2024).

After this period, the disinfected effluent was transferred to four photobioreactors (Fig. 1). In each photobioreactor, 150 L of the treated effluent and 30 L of *S. acuminatus* inoculum were added ( $5 \times 10^7$  cells/mL). The cultivation lasted 7 days, and at the end, the biomass was initially harvested by filtration through a 7  $\mu\text{m}$  polyethylene mesh and later concentrated using a centrifuge. The biomass obtained was frozen in liquid nitrogen, stored in an ultrafreezer at  $-80^\circ\text{C}$ , and then lyophilized.

This biomass production process was repeated twice to ensure sufficient biomass for laboratory analysis and the formulation of the experimental diets. During cultivation, temperature, pH (Hanna HI98194 multiparameter probe), and photosynthetically active radiation (LI-250 photometer) were monitored daily.

#### Growth of microalgae *S. acuminatus*

A 250 mL aliquot was taken daily from each photobioreactor to monitor the growth curve, the day of maximum cell density (MCD), and the specific growth rate ( $\mu$ ; Eq. 1), which represents the cell growth rate during the exponential phase as a function of time.

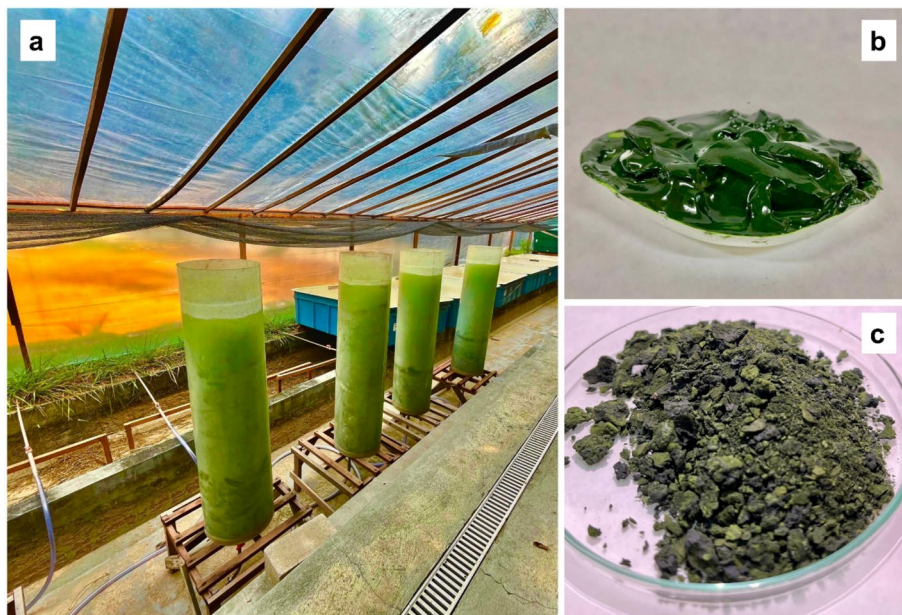


**Fig. 1** **a** Photobioreactor (1.80 m×40 cm Ø). **b** Biomass concentrated after centrifugation. **c** Freeze-dried biomass

$$\mu = \frac{\ln(N_t)/\ln(N_0)}{(t)} \quad (1)$$

In which  $N(t)$  is the final number of cells ( $\text{mL}^{-1}$  cell);  $N_0$  is the initial number of cells ( $\text{mL}^{-1}$  cell);  $t$  is the time interval between  $N_0$  and  $N_t$ .

The biomass production on a dry weight basis was determined at the end of each cultivation cycle from the dry biomass obtained and the total volume cultivated, using the equation:

Yield (g) =  $C_f \times V$  where  $C_f$  is the concentration of final biomass (g/L) and  $V$  is the volume of the cultivation (L).

Daily biomass productivity (dry weight, g/day) was calculated according to the equation:

Daily productivity = total biomass produced (g)/growing time (days).

## Fish feeding experiment with feeds containing *S. acuminatus*

### Processing of experimental feed

From the biomass obtained, five experimental diets were formulated to be approximately isoenergetic ( $17.22 \text{ MJ kg}^{-1}$ ) and isoproteic (32.2% crude protein), meeting the nutritional requirements of juvenile *C. macropomum* (Santos et al. 2021) (Table 1). The feeds

**Table 1** Ingredients and composition of the experimental diets (on dry matter basis)

| Ingredients (%)                         | Experimental diets |       |       |       |       |
|-----------------------------------------|--------------------|-------|-------|-------|-------|
|                                         | 0%                 | 1%    | 2%    | 3%    | 5%    |
| Soybean meal                            | 39.03              | 38.03 | 37.03 | 36.03 | 34.03 |
| Corn grain                              | 27.80              | 27.80 | 27.80 | 27.80 | 27.80 |
| Meat and boné meal                      | 14.00              | 14.00 | 14.00 | 14.00 | 14.00 |
| Wheat bran                              | 13.00              | 13.00 | 13.00 | 13.00 | 13.00 |
| <i>S. acuminatus</i>                    | -                  | 1.00  | 2.00  | 3.00  | 5.00  |
| Poultry viscera meal                    | 2.50               | 2.50  | 2.50  | 2.50  | 2.50  |
| Blood meal                              | 1.75               | 1.75  | 1.75  | 1.75  | 1.75  |
| Vit & Min premix <sup>a</sup>           | 1.00               | 1.00  | 1.00  | 1.00  | 1.00  |
| Dicalcium phosphate                     | 0.30               | 0.30  | 0.30  | 0.30  | 0.30  |
| Sodium Chloride                         | 0.10               | 0.10  | 0.10  | 0.10  | 0.10  |
| BHT                                     | 0.02               | 0.02  | 0.02  | 0.02  | 0.02  |
| L-Lysine                                | 0.20               | 0.20  | 0.20  | 0.20  | 0.20  |
| DL-Methionine                           | 0.30               | 0.30  | 0.30  | 0.30  | 0.30  |
| <b>Proximate composition and energy</b> |                    |       |       |       |       |
| Crude protein (%)                       | 33.88              | 33.97 | 34.24 | 34.02 | 34.23 |
| Crude Lipids (%)                        | 5.21               | 5.14  | 4.8   | 4.95  | 5.06  |
| Crude Ash (%)                           | 8.45               | 8.50  | 8.54  | 8.59  | 8.68  |
| Crude fiber (%)                         | 5.21               | 4.96  | 4.99  | 5.37  | 4.87  |
| Gross energy (MJ/kg)                    | 17.0               | 16.8  | 16.9  | 16.1  | 17.2  |
| $\Sigma$ n-3 (%)                        | 0.14               | 0.15  | 0.14  | 0.16  | 0.18  |
| $\Sigma$ n-6 (%)                        | 1.71               | 1.68  | 1.54  | 1.59  | 1.59  |

<sup>a</sup>Vitamin and mineral mix (Nutron©) per kg of product: folic acid (250 mg), pantothenic acid (5.000 mg), antioxidant (600 mg), biotin (125 mg), cobalt (25 mg), copper (2.000 mg), iron (13.820 mg), iodine (100 mg), manganese (3.750 mg), niacin (5.000 mg), selenium (75 mg), vitamin A (1.000.000 IU), vitamin B1 (1250 mg), vitamin B12 (3.750 mg), vitamin B2 (2.500 mg), vitamin B6 (2.485 mg), vitamin C (28.000 mg), vitamin D3 (500.000 IU), vitamin E (28.000 IU), vitamin K3 (500 mg), zinc (17.500 mg). The values for omega-3 and omega-6 refer to the percentage of total lipids

consisted of a control feed, without addition of microalgae (SA0%), and four feeds containing increasing inclusion levels of SA (SA1%, SA2%, SA3%, and SA5%) (Fig. 2).

All vegetable-based ingredients were mixed in a horizontal mixer and then ground in a hammer mill (Vieira, MCS 280) using a 0.5 mm sieve. Then, the mixture was hydrated to 25% moisture with water at 90 °C in a preconditioner and extruded at 100 °C in a single screw extruder (MX-80, INBRAMAQ®) to produce the 3.0 mm pellets. The pellets were dried in a forced air circulation oven at 55 °C until the moisture content was below 10%, then ground again in a hammer mill (Vieira, MCS 280) with a 0.5 mm sieve and mixed with the ingredients of animal origin and additives in a horizontal mixer. The mixture was hydrated to 35% moisture with water at room temperature and cold extruded at 20 °C in a single screw extruder (ITALVISA, P55), forming micropellets of 1.5 mm. This second extrusion aimed to produce pellets of a suitable size for the fish's mouth. The pellets were dried in a forced air circulation oven at 55 °C until they reached a moisture content that was below 10%.

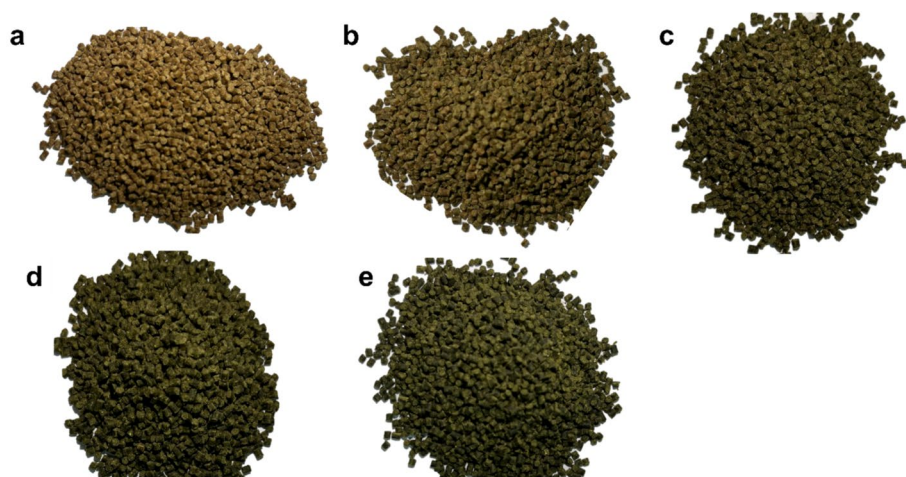


**Fig. 2** Experimental diets: **a** SA0%, **b** SA1%, **c** SA2%, **d** SA3%, and **e** SA5%

### Feeding protocol and growth performance

This study was approved by the Ethics Committee on Animal Experimentation and Research of the National Institute for Amazonian Research (CEUA/INPA), Manaus, Amazonas, Brazil. No: 103/2025/SEI-INPA.

The experiment was conducted using a completely randomized design with five treatments (feeds: control, SA1%, SA2%, SA3%, and SA5%) and three replications, for a total of 15 experimental units. Each experimental unit consisted of a tank (150 L) with 20 juvenile *C. macropomum* ( $1.9 \pm 0.01$  g;  $4.9 \pm 0.25$  cm). The tanks are part of a water recirculation system with mechanical filtration, individual aeration, and natural photoperiod (12:12). Every 3 days, the water temperature ( $29 \pm 2$  °C), dissolved oxygen ( $04 \pm 45$  mg L<sup>-1</sup>), pH (min 6.14. max 6.75), total ammonia ( $0.48 \pm 0.31$  mg L<sup>-1</sup>), and nitrate ( $2.12 \pm 0.14$  mg L<sup>-1</sup>) were monitored. During the experiment, all the water quality parameters remained within the comfort range for juvenile *C. macropomum* (de Farias Lima et al. 2019). The food was divided into two daily meals (8:00 and 17:00), the daily amount offered corresponded to 5% based on the total biomass of each experimental unit during the first 15 days, and 7% until the end of the experiment (5 weeks). At the end of the experiment, the fish were fasted for 24 h and final biometrics were performed to calculate growth performance.

- Weight gain (g) = final weight – initial weight (WG)
- Feed conversion ratio = feed offered/weight gain (FCR)
- Relative growth rate (%/day) =  $(e.g.^{-1}) \times 100$ ; e = Euler number.  $g = [(\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{number of days of the experiment}]$  (RGR)
- Protein efficiency rate (%) = weight gain (g)/protein consumption (g)  $\times 100$  (PER)
- Protein retention rate (%) =  $(\text{final body weight g} \times \text{final total body protein \%}) - (\text{initial body weight g} \times \text{initial total body protein \%}) / \text{protein consumed g} \times 100$  (PRR)
- Final biomass (kg) = final number of fish  $\times$  average final weight (FB)
- Condition factor =  $100 \times (\text{body weight} / \text{total length}^3)$  (CF)
- Survival (%) =  $(\text{final number of fish} \times 100) / \text{initial number of fish}$ .

At the end of the trial, the fish were caught with the aid of a net and anesthetized with clove oil (eugenol), at a concentration of  $20 \text{ mg L}^{-1}$ , according to the protocol described by Inoue et al. (2011). Then, all the fish were measured and weighed individually. Blood samples were collected from the caudal vein of three fish per experimental unit, using 3 mL syringes previously washed with 5% EDTA solution, for hematological and biochemical analyses. Subsequently, the fish were euthanized via spinal cord rupture, in accordance with the CONCEA guidelines (2018).

For the analysis of proximate body composition, nine fish from each experimental unit were stored in a freezer ( $-20^\circ\text{C}$ ). To determine the proximate and fatty acid composition of the fillet, 11 fish from each experimental unit were filleted, and the fillets were immediately frozen with liquid nitrogen and stored in a freezer at  $-20^\circ\text{C}$  until the time of the analyses.

### Proximate and fatty acid composition analyses

The proximate composition of the diets and fish carcass were determined and analyzed according to AOAC (2010). Moisture content was determined by oven-drying at  $105^\circ\text{C}$  until constant weight; crude lipids by solvent extraction (hexane) for 6 h (Soxhlet); ash content by incineration in a muffle furnace at  $500^\circ\text{C}$  for 4 h; and crude protein (CP) by the micro-Kjeldahl method, correcting total nitrogen content by multiplying by the factor 6.25. The crude energy of the samples was determined using a bomb calorimeter (IKA, 2600).

Fatty acids were analyzed following the AOAC (2005; method 996.06) procedures. Lipids extracted from the dried microalgal biomass were converted into fatty acid methyl esters (FAMES) by transesterification using boron trifluoride in methanol ( $\text{BF}_3\text{--MeOH}$ ). The FAMES were analyzed by gas chromatography equipped with a flame ionization detector (GC-FID). Separations were performed on a fused-silica capillary column (e.g.,  $30 \text{ m} \times 0.25 \text{ mm i.d.}$ ,  $0.25 \mu\text{m}$  film thickness). Hydrogen was used as the carrier gas at a constant flow rate of approximately  $1.0 \text{ mL min}^{-1}$ . The injector and detector temperatures were set at  $250^\circ\text{C}$  and  $280^\circ\text{C}$ , respectively. The oven temperature program was as follows: initial temperature of  $140^\circ\text{C}$ , held for 5 min; increased at a rate of  $4^\circ\text{C min}^{-1}$  to  $240^\circ\text{C}$ ; and held for 15 min. Samples ( $1 \mu\text{L}$ ) were injected in split mode (split ratio 1:50). Individual fatty acids were identified by comparison of their retention times with those of a commercial FAME standard mixture.

### Hematological and biochemical parameters

Hematocrit (Ht%) was determined using the microhematocrit method. differential and total counts of leukocytes and thrombocytes were obtained using the indirect method using blood smears stained with May-Grunwald-Giemsa stain. Erythrocytes ( $\text{RBC} \times 10^6 \text{ cell } \mu\text{L}^{-1}$ ) were counted on a hemocytometer ( $10 \mu\text{L}$  of blood,  $2.0 \text{ mL}$  of citrate formaldehyde). The glucose level of the whole blood was determined with a blood glucose meter (Accumed-Glicomed) immediately after blood sampling.

Blood plasma was collected after centrifugation of whole blood at  $3.000 \times g$  for 10 min at  $4^\circ\text{C}$ . These samples were used to analyze the following biochemical variables according to the protocol suggested by the manufacturer (Labtest): total protein (TP), albumin (AB), globulin (GB), triglycerides (TG), cholesterol (CL). Absorbance readings were taken on a spectrophotometer (HACH, DR 6000).

## Statistical analysis

All the data were previously evaluated for normality and homoscedasticity using the Shapiro–Wilk and Levene methods, respectively. Comparisons between treatments were performed using one-way ANOVA followed by Tukey's post-hoc test when necessary. The significance level adopted was  $\alpha=0.05$  (ZAR 1999). All statistical analyses were performed using Jamovi version 2.3.28.

## Results

### Biomass production from *S. acuminatus* and nutritional composition of the biomass

During the experimental period, the physicochemical parameters of the culture medium remained within suitable ranges for *Scenedesmus acuminatus* growth. The average temperature was  $30.1 \pm 1.4$  °C in Cycle 1 and  $31.2 \pm 0.6$  °C in Cycle 2. The pH ranged from 7.52 to 8.80 in Cycle 1 and from 7.30 to 8.21 in Cycle 2, indicating a slightly alkaline medium in both cycles.

The mean irradiance was  $758.4 \pm 10$   $\mu\text{mol s}^{-1} \text{m}^{-2}$  during Cycle 1 and  $157.9 \pm 42$   $\mu\text{mol s}^{-1} \text{m}^{-2}$  during Cycle 2, reflecting differences in light availability between the experiments. Dissolved oxygen concentrations averaged  $5.65 \pm 1.4$   $\text{mg L}^{-1}$  and  $4.48 \pm 0.6$   $\text{mg L}^{-1}$  in Cycles 1 and 2, respectively.

In both cultivation cycles, *S. acuminatus* exhibited a typical growth curve, with a gradual increase in cell density over the 7-day cultivation period (Fig. 3).

In the first cycle, the maximum cell density was recorded on the seventh day, reaching  $1.24 \times 10^8$  cells/mL. The specific growth rate was  $0.57 \text{ d}^{-1}$ , more pronounced between days 2 and 4, indicating the beginning of the exponential phase. In the second cycle, the maximum cell density also occurred on the seventh day, reaching  $1.12 \times 10^8$  cells/mL. The specific growth rate was  $0.41 \text{ d}^{-1}$ , with greater increment between days 3 and 5.

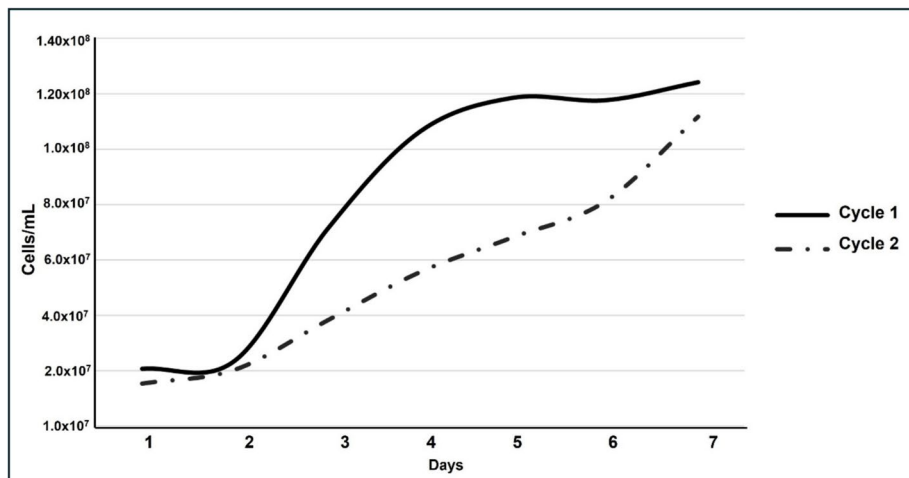


**Fig. 3** Growth of *S. acuminatus* in 2 cycles. The values are the means

Biomass production from *S. acuminatus* varied between the two crop cycles. In the first, 65 g of dry biomass was obtained, resulting in a daily productivity of approximately 9.29 g/day. In the second cycle, conducted using the same volume and time conditions, the dry biomass produced was 55 g, with daily productivity of 7.86 g/day.

The nutritional composition of the biomass of *S. acuminatus* obtained in the crops is presented in Table 2.

### **C. macropomum feeding and growth performance test**

The inclusion of *S. acuminatus* (SA) in the diet of *C. macropomum* did not negatively affect growth performance. The survival rate was 100% in all treatments. Statistical differences were observed only for final body weight, with the highest mean recorded in fish fed the SA3% diet ( $25.2 \pm 3.72$  g), whereas the other growth-related variables did not differ significantly among treatments (Table 3).

The centesimal composition of the whole fish was not negatively influenced by the feeds (Table 4), as no significant differences were observed in the nutritional composition of the fillet (Table 5).

### **Hematological and biochemical parameters**

The fish showed similar values for the variables RBC (erythrocytes), total leukocytes, lymphocytes, and monocytes.

On the other hand, statistical differences in the thrombocyte count were observed ( $p < 0.05$ ) (Table 6). The fish fed SA2% biomass showed higher percentages than the control group ( $p = 0.044$ ), and the SA5% group also differed from the control ( $p = 0.009$ ). Additionally, the SA1% group differed significantly from the SA2% ( $p = 0.005$ ) and SA5% ( $p < 0.001$ ) groups.

Fish fed the SA5% diet had higher neutrophil values in relation to the control group ( $p = 0.026$ ).

In relation to the blood biochemical parameters (glucose, cholesterol, total protein, albumin, triglycerides and globulin), no statistically significant differences were observed among treatments ( $p > 0.05$ ), including cholesterol ( $p = 0.08$ ) (Table 7).

## **Discussion**

### **Growth and nutritional composition of *S. acuminatus* biomass**

The daily productivity observed in this study is within the range reported in experiments conducted with wastewater and natural media (Ansari et al. 2020; Sipaúba-Tavares et al. 2017; Oostlander et al. 2020). This reinforces the challenges of achieving high yields in open or semi-controlled systems while also reflecting real growing conditions with less environmental control. In this context, the results highlight the potential of aquaculture effluent as a viable, sustainable cultivation medium.

The lower productivity recorded in the second cycle compared to the first may be associated with reduced solar radiation exposure, as cultivation occurred during a period of greater cloudiness. This difference highlights the sensitivity of productivity to environmental variations, especially in systems that depend on natural light.

**Table 2** Biochemical composition of biomass *S. acuminatus* biomass (based on dry matter)

| Nutritional composition                           |        |
|---------------------------------------------------|--------|
| Crude protein (%)                                 | 52.1   |
| Crude lipids (%)                                  | 6.5    |
| Crude fibre (%)                                   | 0.37   |
| Gross energy (MJ/kg)                              | 21.2   |
| <i>Fatty acid composition (% of total lipids)</i> |        |
| Palmitic acid (C16:0)                             | 2.07   |
| Oleic acid (C18:1n9c)                             | 1.08   |
| Linoleic acid LA (C18:2n6c)                       | 1.17   |
| Alpha linolenic acid LNA (C18:3n3)                | 1.21   |
| $\sum$ n-3                                        | 1.28   |
| $\sum$ n-6                                        | 1.20   |
| $\sum$ n-9                                        | 1.14   |
| <i>Essential amino acids (%)</i>                  |        |
| Arginine                                          | 2.72   |
| Histidine                                         | 0.74   |
| Lysine                                            | 2.65   |
| Threonine                                         | 2.74   |
| Isoleucine                                        | 1.96   |
| Valine                                            | 2.51   |
| Methionine                                        | 0.80   |
| Phenylalanine                                     | 2.65   |
| Tryptophan                                        | 0.67   |
| <i>Non-essential amino acids (%)</i>              |        |
| Cystine                                           | 0.68   |
| Tyrosine                                          | 1.74   |
| Aspartic acid                                     | 4.2    |
| Glutamic acid                                     | 4.63   |
| Alanine                                           | 4.25   |
| Proline                                           | 2.45   |
| Serine                                            | 1.98   |
| <b>Pigments mg/kg</b>                             |        |
| Beta carotene                                     | 273.97 |
| <b>Vitamins mg/kg</b>                             |        |
| Vitamins E (Tocopherol)                           | 49.04  |
| Vitamins E ( $\beta$ -Tocopherol)                 | 1.56   |
| <b>Minerals</b>                                   |        |
| Calcium %                                         | 0.48   |
| Magnesium %                                       | 0.36   |
| Sodium %                                          | 0.07   |
| Potassium %                                       | 0.45   |
| Copper mg/kg                                      | 99.92  |
| Iron mg/kg                                        | 3.130  |
| Manganese mg/kg                                   | 340.57 |
| Zinc mg/kg                                        | 398.92 |

**Table 3** Growth performance of tambaqui fed diets with increasing levels of biomass of SA for 5 weeks

| Variables | Experimental diets   |                        |                        |                        |                        | <i>p</i> -value |
|-----------|----------------------|------------------------|------------------------|------------------------|------------------------|-----------------|
|           | SA0%                 | SA1%                   | SA2%                   | SA3%                   | SA5%                   |                 |
| IW (g)    | 1.9±0.06             | 1.97±0.06              | 1.97±0.06              | 1.97±0.06              | 1.9±0.06               | -               |
| FW (g)    | 23±4.56 <sup>b</sup> | 23.8±3.45 <sup>a</sup> | 24.1±3.65 <sup>a</sup> | 25.2±3.72 <sup>a</sup> | 23.1±4.38 <sup>b</sup> | 0.03            |
| WG (g)    | 21.1±2.05            | 22.9±0.74              | 22.1±0.47              | 23.2±0.49              | 21.2±1.30              | 0.18            |
| FCR       | 0.79±0.02            | 0.71±0.09              | 0.73±0.01              | 0.67±0.02              | 0.77±0.09              | 0.46            |
| RGR (%)   | 13.2±0.13            | 13.3±0.17              | 13.4±0.08              | 13.7±0.11              | 13.1±0.21              | 0.19            |
| PER (%)   | 3.73±0.14            | 4.1±0.17               | 3.96±0.12              | 4.36±0.07              | 4.03±0.41              | 0.40            |
| PRR (%)   | 58.0±1.24            | 60.1±3.7               | 57.2±2.08              | 63.6±1.84              | 56.9±4.11              | 0.48            |
| FB        | 460±24.8             | 476.3±10               | 481.6±5.89             | 503.3±5.45             | 463±14.9               | 0.28            |
| CF        | 1.79±0.01            | 1.82±0.01              | 1.75±0.01              | 1.84±0.02              | 1.83±0.01              | 0.17            |

Different superscript letters within a row indicate significant differences ( $p < 0.05$ ). Abbreviation: *IW* initial weight, *FW* final weight, *WG* weight gain, *FCR* feed conversion ratio, *RGR* relative growth rate, *PER* protein efficiency rate, *PRR* protein retention rate, *FW* final weight, *FB* final biomass, *CF* condition factor

**Table 4** Proximate whole-body composition of tambaquis fed diets containing graded levels of SA biomass for 5 weeks

| Variables  | Experimental diets |           |           |           |           | <i>p</i> -value |
|------------|--------------------|-----------|-----------|-----------|-----------|-----------------|
|            | SA0%               | SA1%      | SA2%      | SA3%      | SA5%      |                 |
| DM (%)     | 25.6±0.89          | 24.2±0.95 | 24.0±0.36 | 24.3±0.66 | 24.3±0.87 | 0.60            |
| CP (%)     | 60.7±1.13          | 59.7±0.29 | 59.9±0.71 | 60.1±0.78 | 59.1±2.23 | 0.94            |
| AS (%)     | 13.2±0.82          | 13.9±0.14 | 12.9±0.80 | 14.0±0.69 | 13.5±0.69 | 0.74            |
| EE (%)     | 20.3±0.62          | 20.8±1.21 | 21.0±0.98 | 20.4±0.29 | 20.5±1.18 | 0.98            |
| GE (MJ/kg) | 17.8±14            | 17.7±39   | 18±91     | 17.7±86   | 17.7±21   | 0.95            |

*DM* dry matter, *CP* crude protein, *As* ashes, *EE* ether extract, *GE* gross energy

The nutritional composition of the *S. acuminatus* biomass showed crude protein (52.1% dry weight) and lipid (6.5% dry weight) contents within the ranges typically reported for the genus *Scenedesmus*, which generally presents 40–60% protein and 5–25% lipids depending on cultivation conditions (Tomás-Almenar et al. 2018; Koley et al. 2019; Devi et al. 2022). Although total lipid content was at the lower end of this range, it remained within expected values and did not indicate nutritional depletion associated with effluent-based cultivation. Previous studies have shown that biochemical composition can vary with environmental and nutritional factors; however, the values obtained here fall within the standard ranges described for this species. Previous work has shown that the biochemical composition of biomass can vary with environmental and nutritional factors during cultivation. In the present study, using treated effluent as a culture medium did not cause significant losses of the main nutritional constituents, reinforcing its potential as a lower-cost alternative compared with conventional media.

The crude protein and lipid contents obtained confirm that the cultivation environment did not compromise the nutritional value of the biomass, corroborating that the genus *Scenedesmus* is an excellent candidate for bioremediation of effluent and for use of its

**Table 5** Proximate fillet composition of tambaquis fed diets containing graded levels of SA biomass for 5 weeks

| Variables  | Experimental diets |              |              |              |              | <i>p</i> -value |
|------------|--------------------|--------------|--------------|--------------|--------------|-----------------|
|            | SA0%               | SA1%         | SA2%         | SA3%         | SA5%         |                 |
| PB (%)     | 90.11 ± 0.33       | 90.18 ± 0.29 | 90.32 ± 0.52 | 90.19 ± 0.35 | 90.48 ± 0.29 | 0.95            |
| EE (%)     | 3.90 ± 0.45        | 3.84 ± 0.61  | 4.6 ± 0.21   | 4.57 ± 0.48  | 3.15 ± 0.55  | 0.26            |
| C16:0      | 0.87 ± 0.18        | 0.85 ± 0.23  | 1.02 ± 0.07  | 1.02 ± 0.20  | 0.70 ± 0.19  | 0.32            |
| C18:0      | 0.52 ± 0.11        | 0.54 ± 0.13  | 0.63 ± 0.06  | 0.64 ± 0.13  | 0.43 ± 0.13  | 0.40            |
| C18:1n9c   | 1.14 ± 0.22        | 1.09 ± 0.30  | 1.32 ± 0.09  | 1.27 ± 0.22  | 0.88 ± 0.26  | 0.30            |
| C18:2n6c   | 0.52 ± 0.11        | 0.50 ± 0.14  | 0.60 ± 0.04  | 0.60 ± 0.10  | 0.41 ± 0.13  | 0.36            |
| C20:4n6    | 0.27 ± 0.05        | 0.27 ± 0.09  | 0.31 ± 0.02  | 0.32 ± 0.04  | 0.21 ± 0.07  | 0.44            |
| C22:6n3    | 0.08 ± 0.01        | 0.08 ± 0.03  | 0.11 ± 0.01  | 0.11 ± 0.02  | 0.08 ± 0.03  | 0.38            |
| MUFA (%)   | 1.29 ± 0.14        | 1.25 ± 0.20  | 1.50 ± 0.05  | 1.44 ± 0.14  | 1.00 ± 0.17  | 0.24            |
| PUFA (%)   | 1.06 ± 0.12        | 1.05 ± 0.18  | 1.27 ± 0.06  | 1.28 ± 0.12  | 0.88 ± 0.16  | 0.29            |
| SFA (%)    | 1.55 ± 0.18        | 1.53 ± 0.23  | 1.82 ± 0.08  | 1.84 ± 0.21  | 1.25 ± 0.20  | 0.29            |
| Σn-3 (%)   | 0.11 ± 0.01        | 0.12 ± 0.02  | 0.15 ± 0.01  | 0.16 ± 0.01  | 0.12 ± 0.02  | 0.21            |
| Σn-6 (%)   | 0.90 ± 0.11        | 0.89 ± 0.15  | 0.74 ± 0.28  | 1.07 ± 0.10  | 0.73 ± 0.13  | 0.62            |
| EB (MJ/kg) | 17.0 ± 0.01        | 17.1 ± 0.10  | 17.9 ± 0.08  | 17.1 ± 0.30  | 17.7 ± 0.25  | 0.40            |

*CP* crude protein, *EE* ethereal extract, *MUFA* monounsaturated fat, *PUFA* polyunsaturated fat, *SFA* saturated fat; *GE* gross energy

**Table 6** Hematological parameters of tambaqui juveniles fed diets with increasing levels of SA biomass for 5 weeks

| Variables                                | Experimental diets       |                          |                          |                           |                           | <i>p</i> -value |
|------------------------------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|-----------------|
|                                          | SA0%                     | SA1%                     | SA2%                     | SA3%                      | SA5%                      |                 |
| RBC × 10 <sup>6</sup>                    | 1.07 ± 0.07              | 0.95 ± 0.19              | 1.18 ± 0.29              | 1.01 ± 0.27               | 1.04 ± 0.12               | 0.36            |
| LT (× 10 <sup>4</sup> μL <sup>-1</sup> ) | 15.5 ± 6.39              | 13.9 ± 4.14              | 16.5 ± 7.39              | 12.2 ± 3.45               | 13.2 ± 5                  | 0.69            |
| T (× 10 <sup>4</sup> μL <sup>-1</sup> )  | 9.23 ± 2.81 <sup>a</sup> | 7.53 ± 3.31 <sup>a</sup> | 15.1 ± 3.58 <sup>b</sup> | 10.6 ± 4.52 <sup>ab</sup> | 16.3 ± 6.39 <sup>b</sup>  | 0.05            |
| % Lymphocyte                             | 67.1 ± 7.9               | 70.1 ± 7.8               | 62 ± 10                  | 67.7 ± 8.4                | 61.1 ± 6.8                | 0.12            |
| % Neutrophil                             | 8.22 ± 3.34 <sup>a</sup> | 8.94 ± 4.88 <sup>a</sup> | 8.33 ± 6.49 <sup>a</sup> | 12.78 ± 5.25 <sup>a</sup> | 15.39 ± 3.63 <sup>b</sup> | 0.05            |
| % Monocyte                               | 23.6 ± 7                 | 20.7 ± 6.3               | 28.3 ± 7.9               | 19.3 ± 8.8                | 21.6 ± 5                  | 0.20            |

Different superscript letters within a row indicate significant differences (*p* < 0.05). Abbreviations. *RBC* red blood cells count, *LT* total leukocytes, *T* thrombocytes

biomass as an ingredient in animal feed of Tomás-Almenar et al. (2018). This compositional stability indicates viability for applications in animal nutrition and bioproduct development. In addition, the results suggest that it is possible to maintain the biomass's nutritional profile even under less controlled environmental conditions.

Overall, the findings clearly demonstrate the potential of aquaculture effluent as an alternative medium for microalgae cultivation, associating valorization of waste with value-added biomass production. This approach can not only reduce production costs but also provide environmental benefits by decreasing the nutrient load discharged into the environment. Future investigations should prioritize elemental characterization of the effluent

**Table 7** Biochemical parameters of plasma from tambaqui juveniles fed diets with increasing levels of SA biomass for 5 weeks

| Variables             | Experimental diets |             |            |             |             | <i>p</i> -value |
|-----------------------|--------------------|-------------|------------|-------------|-------------|-----------------|
|                       | SA0%               | SA1%        | SA2%       | SA3%        | SA5%        |                 |
| Glucose (mg/dL)       | 124 ± 8            | 129 ± 10    | 152 ± 6    | 124 ± 14    | 138 ± 5     | 0.91            |
| Cholesterol (mg/dL)   | 45.48 ± 9.1        | 68.8 ± 14.8 | 44 ± 2.8   | 76.7 ± 13.7 | 45.3 ± 5.06 | 0.08            |
| Total protein (mg/dL) | 2.50 ± 0.20        | 2.07 ± 0.10 | 2.82 ± 1.2 | 2.51 ± 0.28 | 2.39 ± 0.32 | 0.32            |
| Albumin (g/dL)        | 1.51 ± 0.40        | 1.30 ± 0.17 | 2.34 ± 0.7 | 1.41 ± 0.18 | 1.16 ± 0.02 | 0.28            |
| Triglycerides (mg/dL) | 227.3 ± 69.26      | 177.10 ± 6  | 196.81 ± 7 | 165.90 ± 2  | 142.59 ± 7  | 0.85            |
| Globulin (g/dL)       | 1.39 ± 0.25        | 0.86 ± 0.16 | 1.7 ± 0.45 | 0.96 ± 0.35 | 1.42 ± 0.42 | 0.34            |

to identify limiting nutrients and thereby optimize the final biomass composition for its intended use.

### Growth performance of *C. macropomum*

The high survival rate and low feed conversion ratio (FCR) values suggest that the inclusion of *Scenedesmus acuminatus* did not compromise the feed's palatability. Although palatability was not directly measured, diets containing microalgae showed better feed efficiency than the control treatment. FCR values were  $0.71 \pm 0.09$  (SA1%),  $0.73 \pm 0.01$  (SA2%),  $0.67 \pm 0.02$  (SA3%), and  $0.77 \pm 0.09$  (SA5%), compared to  $0.79 \pm 0.02$  in the control. The best feed efficiency was observed with 3% inclusion ( $0.67 \pm 0.02$ ), indicating greater nutrient utilization.

Similar results were reported by Silva et al. (2023), who, when using *Scenedesmus obliquus* in a multitrophic system with tilapia (*Oreochromis niloticus*) and shrimp (*Litopenaeus vannamei*), did not observe a negative effect on feed conversion ratio (FCR) and even found higher survival rates for both species. Tomás-Almenar et al. (2018), when evaluating the partial replacement of fishmeal with *Scenedesmus almeriensis* in the diet of rainbow trout (*Oncorhynchus mykiss*), observed reduced growth and FCR values lower than the control at the highest inclusion levels (5% and 10%), but still within the range considered normal for the species at this growth stage. In general, the literature indicates that moderate levels of microalgae biomass inclusion tend to maintain or improve feed efficiency, whereas excessively high levels can compromise zootechnical performance, as observed by Dallaire et al. (2007) with *Spirulina* sp. inclusion levels greater than 20% in the diet of *Oreochromis* sp. Therefore, the effects of including microalgae in feed cannot be generalized, as they depend on the microalgae species, the fish species, the animals' developmental stage and size, and the nutritional composition and digestibility of the biomass used. These factors are crucial for correctly adjusting inclusion levels and achieving positive zootechnical results.

In the present study, the inclusion of up to 5% of *S. acuminatus* did not compromise fish survival, indicating good compatibility of the ingredient with the target species, in addition to greater weight gain compared to the control feed. These results suggest that *S. acuminatus* biomass grown in aquaculture effluent maintains nutritional and functional properties suitable for commercial formulations, without compromising feed acceptance.

The increase in thrombocyte counts in the SA2% and SA5% feeds suggests a stimulus for platelet hematopoiesis, possibly associated with the presence of bioactive compounds in *S. acuminatus*, such as polyunsaturated fatty acids, carotenoids, and polysaccharides, which are recognized for their modulating action on the immune response. Similar results were reported by Abdel-Tawwab et al. (2022) and Skalli et al. (2020) in tilapia and trout (*Oncorhynchus mykiss*), respectively, fed diets supplemented with *S. quadricauda* and *Scenedesmus* sp., respectively, which were attributed to the strengthening of immunocompetence in the face of environmental and disease challenges.

The increase in the percentage of neutrophils in fish fed the SA3% and SA5% diets may indicate activation of the innate immune system, since neutrophils are key phagocytic cells involved in nonspecific defense responses. A similar pattern was reported by Ferreira et al. (2024) in *Colossoma macropomum* fed *Chlorella* and *Spirulina*, supporting the hypothesis that microalgae can act as functional ingredients that modulate basal immunity. However, although the magnitude of the neutrophil increase was statistically significant, it was biologically moderate, as it was not accompanied by changes in total leukocytes, lymphocytes, or monocytes. This indicates a physiological immunomodulatory adjustment rather than an exacerbated inflammatory response. The absence of leukocyte imbalance suggests that the fish were not under immunological stress or a pathological challenge, and that microalgal inclusion did not induce detrimental inflammatory activation, a desirable condition from a growth and health perspective.

The maintenance of plasma biochemical parameters (glucose, cholesterol, total proteins, albumin, globulins, and triglycerides) within the physiological ranges reinforces the absence of adverse effects on hepatic and protein metabolism, evidencing the metabolic stability of the animals regardless of the level of inclusion of *S. acuminatus*. Similar results were reported by Maryshamya et al. (2019) and Silva et al. (2023), who also observed no biochemical changes in *Danio rerio* and tilapia fed on *S. quadricauda* and *S. obliquus*, respectively.

The results indicate that the biomass of *S. acuminatus* cultivated in aquaculture effluent did not compromise the health of the juvenile *C. macropomum*, which maintained stable hematological and biochemical parameters, with evidence of positive activation of the immune system. These findings reinforce the potential of microalgae as a functional and safe ingredient, in line with the trend of adopting more sustainable inputs in aquaculture.

The stability in the values of the dry matter and crude protein, ether extract, ash, and crude energy among the feed groups suggests that the inclusion of *S. acuminatus* in the feed, even at levels of up to 5%, did not compromise the body composition of the juvenile *C. macropomum*. This constancy indicates that the microalgae was well assimilated, without causing changes in the nutritional parameters evaluated.

The crude protein levels observed were within the range commonly reported in the literature for *C. macropomum* fed with conventional feeds (Rodrigues et al. 2024). This demonstrates that the inclusion of microalgae did not compromise protein deposition in the animals' bodies and that the formulation and quantity offered during the study adequately met the nutritional requirements of the species.

Likewise, the stability in the contents of ether extract and crude energy between treatments indicates that the inclusion of *S. acuminatus* did not influence lipid or energy deposition in fish, thus not compromising their development. This stability may be associated with the lipid profile of the microalgae biomass, which is characterized by the presence of unsaturated fatty acids, but has a low total lipid content, which possibly limits its direct impact on the accumulation of body fat.

The inclusion of *S. acuminatus* in *C. macropomum* diets demonstrated that microalgae can function as a nutritional ingredient while adding potential commercial value. Polyunsaturated fatty acids, particularly omega-3, were numerically higher in the SA3% treatment, representing a 45.45% relative increase compared with the control. The omega-3 content rose from 0.11% in the control fillet to 0.16% in the SA3% group, corresponding to a net increase of 0.05 percentage points. Although this difference was not statistically significant ( $p=0.21$ ), even modest increases in dietary omega-3 intake may be nutritionally meaningful when consumed regularly (WHO 2015).

Interestingly, the higher enrichment observed at 3% inclusion did not continue at 5%, suggesting that fatty acid deposition in tambaqui muscle may not respond in a strictly linear manner to increasing dietary supply. In fish, the incorporation of dietary lipids into tissues involves selective absorption, transport, enzymatic conversion, and oxidation processes. These pathways can impose physiological limits on the amount of additional omega-3 ultimately retained in the fillet (Sargent et al. 2002; Tocher 2003). In other words, simply increasing the dietary input does not necessarily guarantee proportional tissue enrichment.

Another factor likely influencing the observed response is the dietary fatty acid balance. The n-6:n-3 ratios in the experimental diets ranged from 8.8 to 12.2, values that exceed the optimal range described for tambaqui by Paulino et al. (2018), who reported that LNA/ALA ratios between 3.9 and 5.6 maximize EPA and DHA deposition. When n-6 levels are relatively high, they compete with n-3 fatty acids for the same desaturase and elongase enzymes involved in long-chain PUFA synthesis. Under these conditions, part of the dietary ALA may be preferentially oxidized or incorporated without further elongation, which could help explain the moderate, non-significant increase in fillet omega-3 at higher inclusion levels. Adjusting the dietary fatty acid balance, rather than increasing the inclusion rate alone, may be a more effective strategy for optimizing n-3 deposition in tambaqui.

From a commercial perspective, even this qualitative modulation of the lipid profile is relevant. The omega-3 enrichment did not alter total lipid content, as ether extract values were similar across treatments. This indicates that the diets promoted a shift in fatty acid composition rather than an increase in overall fat accumulation, a characteristic that may appeal to consumers seeking healthier products. At the same time, the maintenance of protein and energy levels confirms that nutritional adequacy was preserved.

Finally, cultivating *S. acuminatus* in fish-farming effluent proved to be a viable and sustainable approach for producing biomass with adequate nutritional value. Its safe inclusion in tambaqui diets reinforces the potential of microalgae as a functional ingredient within more circular and resource-efficient aquaculture systems.

## Conclusion

The results demonstrate that *Scenedesmus acuminatus* biomass can be successfully cultivated using fish farming effluents and incorporated into diets for *C. macropomum* at inclusion levels up to 5% without compromising growth performance, survival, or fillet nutritional quality. Although no statistically significant differences were observed among treatments, the 3% inclusion level numerically enhanced fillet omega-3 content, indicating potential for nutritional enrichment. Overall, the integration of effluent valorization with microalgal biomass production and dietary application represents a promising strategy to enhance sustainability and support circular bioeconomy approaches in aquaculture.

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

## Declarations

**Competing interests** The authors declare no competing interests.

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