Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Hematological and anthelminthic responses of tambaqui (*Colossoma macropomum*) supplemented with *Artrhospira platensis* and *Chlorella vulgaris*

Respostas hematológicas e antihelmínticas de tambaqui (*Colossoma macropomum*) suplementado com *Artrhospira platensis* e *Chlorella vulgaris*

A. C. dos S. Ferreira[a], A. M. Pacheco[b], C. B. Salomão[b], M. V. F. Moreira[a], M. F. Martins[b], P. V. N. Santos[b], L. Prestes[c], Y. I. C. Furtado[a], M. Tavares-Dias[a,d], A. M. da Silveira-Junior[e] and E. T. O. Yoshioka[a,d*]

[a]Universidade Federal do Amapá – UNIFAP, Programa de Pós-Graduação em Biodiversidade Tropical, Macapá, AP, Brasil.
[b]Universidade do Estado do Amapá – UEAP, Colegiado de Engenharia de Pesca, Macapá, AP, Brasil.
[c]Universidade Federal do Pará – UFPA, Núcleo de Ecologia Aquática e Pesca da Amazônia, Belém, Pará, Brasil.
[d]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Amapá, Laboratório de Aquicultura e Pesca, Macapá, AP, Brasil.
[e]Universidade Federal do Amapá – UNIFAP, Departamento de Meio Ambiente e Desenvolvimento, Macapá, AP, Brasil.

Abstract

The present study evaluated the hematological, antiparasitic and growth responses in tambaqui (*Colossoma macropomum*) fed with diets supplemented with the microalgae *Arthrospira platensis* and *Chlorella vulgaris* (0%; 10% *A. platensis*; 10% *C. vulgaris*; and 5% *A. platensis*+5% *C. vulgaris*). Tambaqui (n=60, 62.57 ± 8.76 g) were fed for 20 days with experimental diets. Blood samples collection was done to determine hematological parameters, and gills were removed to identify and count monogenetic parasites. Supplementation with *A. platensis* 10% reduced red blood cells count, in consequence mean corpuscular volume and mean hemoglobin concentration increased. Total leukocyte, monocyte, eosinophil, and basophil counts reduced with the use of *A. platensis*. Higher monocytes, eosinophil, and basophil numbers in tambaqui fed with diet supplemented with 10% *C. vulgaris* were observed and may have been due to the presence of immunostimulants in this microalga composition. Reduction on total cholesterol in tambaqui that received both microalgae (*A. platensis* 5%+*C. vulgaris* 5%) may indicate that combined supplementation presented greater benefits to the health for *C. macropomum* than separately. Both microalgae were efficient against monogenetic parasites of tambaqui. Thus, the dietary use of the microalgae *A. platensis* and *C. vulgaris* provided immunostimulant and antiparasitic efficacy in *C. macropomum*.

Keywords: health status, microalgae supplementation, immunostimulant action, cholesterol, monogenea.

Resumo

O presente estudo avaliou as respostas hematológicas, antiparasitária e de crescimento de tambaqui (*Colossoma macropomum*) após alimentação com dietas suplementadas com microalgas *Arthospira platensis* e *Chlorella vulgaris* (0%; 10% *A. platensis*; 10% *C. vulgaris;* e 5% *A. platensis*+5% *C. vulgaris*). Exemplares de tambaqui (n=60, 62,57±8,76 g) foram alimentados por 20 dias com as dietas experimentais. Amostras de sangue foram coletadas para determinação dos parâmetros hematológicos e brânquias foram retiradas para contagem de parasitos monogenéticos. A suplementação com *A. platensis* 10% reduziu o RBC, consequentemente aumentando os valores de VCM e HCM. As contagens de leucócitos (total) e de monócitos, eosinófilos e basófilos reduziram com o uso de *A. platensis*. Números altos de monócitos, eosinófilos e basófilos em tambaqui alimentado com dietas com 10% *C. vulgaris* foram observados e podem ter ocorrido devido à presença de imunoestimulantes na composição da microalga utilizada. Redução dos níveis plasmáticos de colesterol total em tambaqui indicou que a inclusão das duas microalgas (*A. platensis* 5%+*C. vulgaris* 5%) pode indicar que maiores benefícios à saúde do peixe do que quando separadamente suplementada. Ambas mostraram eficácia contra parasitos monogenéticos das brânquias de tambaqui. Assim, conclui-se que o uso das microalgas *C. vulgaris* e *A. platensis* promovem ação imunoestimulante com eficácia antiparasitária em *C. macropomum*.

Palavras-chave: condição de saúde, suplementação com microalgas, ação imunoestimulante, colesterol, monogenea.

*e-mail: eliane.yoshioka@embrapa.br
Received: September 13, 2023 – Accepted: May 10, 2024

1. Introduction

Aquaculture as a sustainable activity produces healthy foods and has social benefits through providing employment and income. Moreover, it is highly profitable and produces high-quality animal protein (Valenti et al. 2021). Considering that the production costs are high, it is very important to seek ingredients for these farmed animals diet that functionally go beyond their nutritional needs. Thus, the use of natural supplements instead of antibiotics has been strongly sought by the aquaculture industry (Abdel-Warith and Elsayed, 2019; Iftikhar and Hashmi, 2021; Rahimi et al. 2022). Within this trend, the use of microalgae biomass such as from *Chlorella* sp. (Trebouxiophyaceae) and *Arthrospira* sp. (Cyanophyceae) has been gaining ground (Valenti et al. 2021), given that it has high nutritional value consisting of approximately 60% protein and 1 to 70% lipids, along with a wide variety of essential minerals and aminoacids (Abdel-Warith and Elsayed, 2019; Silveira Júnior et al., 2019; Alagawany et al., 2021). In addition, microalgae biomass may present beneficial action against infectious and parasitic diseases (Andrade et al., 2018; Bratchkova and Kroumov, 2020; Zielinski et al., 2020). Tambaqui is a native fish species of the Amazon basin that is prominent within Brazilian aquacultural production given that it presents rapid weight gain and is highly appreciated for culinary purposes. However, the major obstacle to expansion of the market for this species is the high production costs mainly relative to feeding and maintenance of fish health (Izel and Melo, 2004; Ribeiro et al., 2016; Aride et al., 2021). Thus, the aim of the present study was to evaluate growth, hematological, and antiparasitic responses of tambaqui (*Colossoma macropomum*) after feeding them with microalgae *Arthrospira platensis* and *Chlorella vulgaris* supplemented diets.

2. Materials and Methods

The present study was authorized by the Embrapa Amapá Ethics Committee for the use of Animals in Experiments (CEUA), protocol nr. 018-CEUA/CPAFAP and it is registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), under number A0D6DC.

2.1. Experimental design and diets

The experiments were carried out at the Aquaculture and Fisheries Laboratory, Embrapa Amapá, Macapá, AP. Tambaqui (*C. macropomum*) specimens (n = 60) of initial mean weight 62.57±8.76 g were randomly divided among 12 experimental tanks (polypropylene water containers of 250 L capacity). Fish were fed with commercial feed containing 40% crude protein (Agronorte Rações, Tocantinópolis, TO, Brazil) four times a day (at 08:00, 11:00, 14:00 and 17:00h) during acclimatization and the experiment period.

The experimental groups of the present study were run in triplicate, as follows: a) Control (without inclusion of microalgae: 0% *C. vulgaris* + 0% *A. platensis*); b) C10, with inclusion of 10% *C. vulgaris*; c) A10, with inclusion of 10% *A. platensis*; d) C5+A5, with inclusion of 5% *C. vulgaris* and 5% *A. platensis*. The microalgae *A. platensis* (batch 202110405, China) and *C. vulgaris* (batch 997734, China), both in powdered form, were acquired commercially in Macapá, AP. The microalgae were analyzed using an optical microscope in the Environmental Sanitation Laboratory, Federal University of Amapá (UNIFAP), Macapá, AP, to confirm the degree of purity of the products. The experimental diets with the following levels of microalgae supplementation were obtained: a) C10, with incorporation of 100 g of *C. vulgaris* per 1 kg of extruded commercial feed (i.e. inclusion of 10% *C. vulgaris*); b) A10, with incorporation of 100 g of *A. platensis* per 1 kg of extruded commercial feed (i.e. inclusion of 10% *A. platensis*); c) C5+A5, with incorporation of 50 g of *C. vulgaris*+50 g of *A. platensis* per 1 kg of extruded commercial feed (i.e. inclusion of 5% *C. vulgaris* and 5% *A. platensis*); and d) Control, consisting of extruded commercial feed without incorporation of any microalgae (0% *C. vulgaris*+0% *A. platensis*). All experimental diets were prepared with a volume of 50 mL of soybean oil. In the case of diets with microalgae, soybean oil was used to incorporate the microalgae into each one kg of extruded commercial fish feed. After experimental diets were homogenized well and left to dry in a chamber heated to 60 °C about for 24 h.

The daily offered feed was equivalent to 5% of the total biomass of each experimental tank, slowly and carefully in order to permit fish quickly capture the granules of ration, avoiding loss of microalgae to water and ensuring the arrival of feed ration (and it supplemented product) to the digestive tract. To analyze centesimal composition of the experimental diets, 100 g of feed from each experimental diet was collected in triplicate (IAL, 2008; AOAC International, 1995; Silva and Queiroz, 2002). Table 1 shows the chemical composition of the experimental diets. During the period of the experiment, the oxygen levels, temperature, and pH of the water were monitored using a multiparametric probe (Hanna, USA, mod. HI 9829, Kyoto, Japan). The following means and standard deviations were obtained: dissolved oxygen, 5.2±1.88 mg/L; temperature, 28.45±1.00 ºC; and pH, 6.8±0.89. The water quality in experimental tanks was adequate for tambaqui farm (Corrêa et al., 2018).

2.2. Growth and hematological analysis

After the feeding period, five tambaqui specimens from each replication (n = 60) were caught and anesthetized with 0.1% benzocaine to minimize the stress of handling. After blood collection, the specimens were euthanized by spinal transection to obtain the total weight and total length; removal and weighing of the to check the hepatosomatic index (HSI=liver weight/body weight x 100).

A blood sample was obtained from each specimen by puncturing the caudal vein with a syringe containing anticoagulant (EDTA 5%). The following hematological parameters were determined: hematocrit (Ht, %), which refers to the percentage of erythrocytes in the blood, obtained through centrifugation of capillary tubes in a microhematocrit centrifuge (Micro Spin, model SPIN

Table 1. Proximal chemical composition (%) of the experimental diets: Control (0% *C. vulgaris*+0% *A. platensis*); C10, 10% supplementation with the microalga *C. vulgaris*; A10, 10% supplementation with the microalga *A. platensis*; and C5+A5, supplementation with % *C. vulgaris* and 5% *A. platensis*.

Parameters	Control	C10	A10	C5+A5
Dry matter (%)	93.86±0.51	96.21±0.25	97.13±0.11	96.93±0.21
Crude Protein (%)	33.19±1.15	36.01±0.45	31.42±3.29	32.92±3.52
Ether extract (%)	4.29±0.58	8.59±0.34	13.14±0.95	11.26±0.57
Ash (%)	13.31±0.22	11.68±0.41	11.57±0.35	11.59±0.15
Fiber (%)	5.92±2.38	7.29±1.85	8.56±1.93	7.96±1.62
Calcium (%)	1.83±0.06	1.71±0.13	1.66±0.21	1.74±0.07
Phosphorous (%)	1.54± 0.07	1.50 ± 0.03	1.42 ± 0.12	1.50 ± 0.01
Iron (%)	515.78±41.94	390.57±46.35	405.54±45.07	413±14.28
Copper (%)	35.22±5.56	24.59±2.77	22.57±2.80	23.48±0.93
Zinc (%)	319.02±36.77	233.79±21.86	218.43±18.68	213.54±4.88
Manganese (%)	68.77±9.96	51.05±3.49	46.46±4.27	48.80±2.91

Data expressed as mean±standard deviation.

1000), followed by reading the results using a reading card (Goldenfarb et al., 1971); hemoglobin concentration (Hb, $g.dL^{-1}$), determined using the cyanmethemoglobin method, with absorbance readings at 540 nm in a spectrophotometer (Biospectro, SP-220) (Collier, 1944); and red blood cells count (RBC, 10^6 μL^{-1}), performed through dilution of blood in a formol-citrate solution, with counting done in a Neubauer chamber under an optical microscope (Boeco, model BOE-01) (Ranzani-Paiva et al., 2013). From these results obtained, the following hematimetric indices were calculated: mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, $g.dL^{-1}$) and mean corpuscular hemoglobin concentration (MCHC, $g.dL^{-1}$) (Ranzani-Paiva et al., 2013). Blood smears were prepared in duplicate and were stained with the May-Grünwald-Giemsa-Wright to obtain total leukocytes, total thrombocytes, and differential leukocytes counts, by indirect method (Ishikawa et al., 2008). After centrifugation of the blood (at 75 G, for 10 minutes) (Centrifuge 5424, Eppendorf, Hamburg, Germany), the plasma obtained was used to determine the total protein, albumin, glucose, total cholesterol, and triglyceride concentrations (Labtest Diagnóstica S.A., Minas Gerais, Brazil). The samples were read using a spectrophotometer (Biospectro SP-220, Curitiba, Paraná, Brazil), at wavelengths specific for each metabolite.

2.3. Parasites identification and quantification

After 20 days of feeding, the gills (n = 60) from each specimen were collected from fish and fixed in 5% formalin, for quantification and identification of the monogeneans. These parasites were prepared for identification using previous recommendations (Eiras et al., 2006). After quantification, the prevalence, mean intensity, and mean abundance were calculated (Bush et al., 1997). The efficacy of each treatment was also calculated, according to Zhang et al. (2014).

2.4. Statistical analysis

All data were previously evaluated to normality and homoscedasticity using Shapiro-Wilk and Levene, respectively, at one-way analysis (ANOVA), with a post-hoc Tukey test. As the data did not follow a normal distribution, the Kruskal-Wallis test was used, and the differences between the medians were compared using Nemeyer post-hoc test All at a significance level of α=0.05 (Zar, 2010). All these statistical analyses were performed in the R Core Team software (2020).

3. Results

During the 20-day period of feeding, fish showed growth through comparing the initial and final weights and the initial and final standard lengths. However, there were no significant differences between the experimental groups. Fish mortality was not observed, with 100% of survival as indicated at Table 2. The HIS was similar ($p > 0.05$) between the experimental groups (Table 2).

The hematological parameters and plasma levels of glucose, total proteins, albumin, total cholesterol, and triglycerides of the tambaqui fed with diets containing different concentrations of microalgae are presented in Table 3. The Ht, Hb and MCHC values did not present significant differences through use of *A. platensis* and *C. vulgaris* in the diets, compared with those fed only with commercial feed. The plasma concentrations of glucose, total proteins and triglycerides were similar ($p > 0.05$) in all the experimental groups. The plasma levels of total cholesterol and albumin of the fish in group A10 increased ($p < 0.05$) in relation to C5+A5. According to the results showed on Table 3, RBC was significantly lower in tambaqui that received 10% *A. platensis* in their diet, compared with all other groups. The MCV of the group A10 was significantly higher than those of the Control group. The MCH of A10 were also higher ($p < 0.05$) than all other treatments.

Table 2. Initial and final weight and length and hepatosomatic index (HSI) of juvenile tambaqui (*Colossoma macropomum*) fed diets supplemented with the microalgae *Arthrospira platensis* and *Chlorella vulgaris*: Control (0% *C. vulgaris*+0% *A. platensis*); C10, 10% supplementation with *C. vulgaris*; A10, 10% supplementation with *A. platensis*; and C5+A5, 5% supplementation with *C. vulgaris* and 5% supplementation with *A. platensis*.

	Control	C10	A10	C5+A5
Initial weight (g)	62.96±8.77[a]	65.07±9.59[a]	59.51±7.47[a]	62.73±9.08[a]
Final weight (g)	110.96±16.57[a]	106.96±0.99[a]	100.5±17.2[a]	98.51±18.54[a]
Initial length (cm)	12.67±0.49[a]	12.77±0.59[a]	12.50±0.42[a]	12.70±0.56[a]
Final length (cm)	15.26±1.00[a]	15.35±0.51[a]	14.91±0.80[a]	14.58±1.10[a]
Fish survival (%)	100	100	100	100
HSI (%)	1.27±0.26[a]	1.37±0.23[a]	1.35±0.20[a]	1.43±0.17[a]

Data expressed as mean±standard deviation. Different letters in line means significant differences ($p<0.05$).

Table 3. Hematological variables and plasmatic levels of glucose, total proteins, albumin, total cholesterol, and triglycerides of juvenile tambaqui (*Colossoma macropomum*) fed diets supplemented with the microalgae *Arthrospira platensis* and *Chlorella vulgaris*: Control (0% *C. vulgaris*+0% *A. platensis*); C10, 10% supplementation with the microalga *C. vulgaris*; A10, 10% supplementation with the microalga *A. platensis*; and C5+A5, supplementation with % *C. vulgaris* and 5% *A. platensis*.

	Control	C10	A10	C5+A5
Ht (%)	28.27±2.15[a]	29.17±3.30[a]	29.23±2.96[a]	29.53±2.94[a]
Hb ($g.dL^{-1}$)	7.50±0.94[a]	7.15±0.72[a]	7.80±0.78[a]	7.73±1.11[a]
RBC (x 10^6 μL^{-1})	1.89±0.22[a]	1.96±0.29[a]	1.70±0.17[b]	1.92±0.20[a]
MCV (fL)	150.66±14.79[b]	152.94±32.69[ab]	173.48±1.68[a]	155.29±21.96[ab]
MCH ($g.dL^{-1}$)	40.07±6.34[b]	37.10±4.96[b]	46.24±5.06 [a]	40.64±7.16[b]
MCHC ($g.dL^{-1}$)	26.53±2.66[a]	24.85±4.11[a]	26.83±2.78[a]	26.17±2.45[a]
Glucose ($mg.dL^{-1}$)	55.33±9.18[a]	57.82±12.69[a]	52.87±12.44[a]	55.41±12.65[a]
Total protein ($g.dL^{-1}$)	3.08±0.34[a]	3.30±0.34[a]	3.55±0.63[a]	3.42±0.61[a]
Albumin ($g.dL^{-1}$)	0.59±0.12[ab]	0.64±0.12[ab]	0.69±0.16[a]	0.52±0.11[b]
Total Cholesterol ($mg.dL^{-1}$)	58.68±12.00[ab]	61.57±11.84[ab]	71.89±11.46[a]	52.63±7.69[b]
Triglycerides ($mg.dL^{-1}$)	156.96±27.67[a]	139.06±21.35[a]	158.23±31.27[a]	145.03±6.21[a]

Data expressed as mean±standard deviation. Different letters in line means significant differences ($p<0.05$). Ht, Hematocrit; Hb, Hemoglobin Concentration; RBC, Red Blood Cells Count; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration.

The total thrombocyte and the total and differential leukocyte counts of the tambaqui are presented in Table 4. The thrombocyte, lymphocyte, and neutrophil and PAS-LG counts did not show statistically significant differences between the experimental groups. The total leukocyte, monocyte, eosinophil, and basophil counts were lower ($p < 0.05$) in the blood of tambaqui fed with *A. platensis* (A10) than the Control and C10 groups.

All fish evaluated, regardless of the diet that they received, were parasitized by monogeneans (*Anacanthorus spathulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni*) (Table 5). The abundance of monogeneans on the gills of *C. macropomum* varied between the treatments. All the treatments with microalgae (C10, A10 and C5+A5) showed antiparasite activity against monogeneans on the gills of the tambaqui and, thus, all the treatments showed good efficacy.

4. Discussion

Tambaqui presented body growth, with weight and length increases. However, *A. platensis* e *C. vulgaris* microalgae dietary supplementations were not the promoter of this growth, since this was similar to Control group. These findings differed from those of Faheem et al. (2022), that reported there was a weight gain of *Ctenopharyngodon idella* fed with diets containing 1 to 5% *A. platensis*. Likewise, Simanjuntak et al. (2018) reported that there was an increase in the total weight of *Osphronemus gouramy* with addition of *A. platensis* at 6 g/kg. Using diets in which 10 to 30% of the fish meal was replaced by *A. platensis*, Abdel-Warith and Elsayed (2019) observe that Nile tilapia grew after 12 weeks. According to those studies, microalgae effects on fish growth are dependent on some factor of the fish species studied such as physiological responses, feeding period used, levels of supplementation and development stage of the fish.

Table 4. Total thrombocytes and total and differential leukocytes count in juvenile tambaqui (*Colossoma macropomum*) fed diets supplemented with the microalgae *Arthrospira platensis* and *Chlorella vulgaris*: Control (0% *C. vulgaris*+0% *A. platensis*); C10, 10% supplementation with the microalga *C. vulgaris*; A10, 10% supplementation with the microalga *A. platensis*; and C5+A5, supplementation with % *C. vulgaris* and 5% *A. platensis*.

	Control	C10	A10	C5+A5
Thrombocytes (x 10^3 μL^{-1})	108.21±30.77^a	145.72±59.78^a	110.99±39.48^a	111.22±31.36^a
Leukocytes (x 10^3 μL^{-1})	197.20±26.99^a	210.36±37.98^a	169.04±18.1^b	193.37±19.75^{ab}
Lymphocytes (x 10^3 μL^{-1})	86.45±25.45^a	103.64±35.10^a	81.44±21.82^a	98.74±24.52^a
Monocytes (x 10^3 μL^{-1})	53.54±12.72^a	49.69±12.75^{ab}	32.19±11.52^c	43.38±19.76^{abc}
Neutrophils (x 10^3 μL^{-1})	33.28±10.75^a	29.11±11.73^a	37.03±11.36^a	33.37±17.72^a
PAS-LG (x 10^3 μL^{-1})	4.83±2.91^a	9.33±7.44^a	12.92±7.55^a	10.63±5.92^a
Eosinophils (x 10^3 μL^{-1})	8.24±3.91^a	8.75±5.54^{ab}	2.37±1.78^c	4.87±3.18^{abc}
Basophils (x 10^3 μL^{-1})	10.86±5.32^a	10.02±5.79^a	3.10±2.16^b	2.73±2.79^b

Data expressed as mean±standard deviation. Different letters in line means significant differences ($p<0.05$). PAS-LG, Granular Leukocyte PAS positive.

Table 5. Parasitological indices and efficacy of treatment of monogeneans on the gills of juvenile tambaqui (*Colossoma macropomum*) fed diets supplemented with the microalgae *Arthrospira platensis* and *Chlorella vulgaris*: Control (0% *C. vulgaris*+0% *A. platensis*); C10, 10% supplementation with the microalga *C. vulgaris*; A10, 10% supplementation with the microalga *A. platensis*; and C5+A5, supplementation with % *C. vulgaris* and 5% *A. platensis*.

	Control	C10	A10	C5+A5
Prevalence (%)	100	100	100	100
Mean Intensity	37.3±10.2^a	10.9±8.5^b	12.1±7.9^b	15.1±3.2^b
Mean Abundance	37.3±10.2^a	10.9±8.5^b	12.1±7.9^b	15.1±3.2^b
Efficacy (%)	-	70.8	67.6	59.5

Data expressed as mean±standard deviation. Different letters in line of abundance means significant difference ($p<0.05$).

The liver is an important organ for a variety of metabolic processes, and the HSI can therefore be used as a biomarker at organ level, due to it increasing under stress situations (Morado et al., 2017). The microalgae supplementation used in the present study did not promote changes in HSI of tambaqui and it can be presumed that none of the diets, containing either of the microalgae evaluated, caused any damage to the liver of these fish. Similar results after fishmeal replacement with inclusions from 2.5% up to 10% of *Nannochloropsis* sp. in the diets were reported for juvenile turbot (*Scophthalmus maximus* L.) by Quiao et al. (2019).

Hematological variables of farmed fish may provide important information about the nutritional status and health condition of the fish (Tavares-Dias et al., 2009; Raji et al., 2018; Faheem et al., 2022). For example, the RBC is an important indicator of anemia and other dysfunctions (Ranzani-Paiva et al., 2013), when assessed with hematocrit and hemoglobin concentration. The lower RBC observed in the tambaqui fed with inclusion of 10% *A. platensis*, compared with all the other experimental groups, occurred probably in consequence of the MCV and MHC increases. Nevertheless, in contrary Abdel-Warith and Elsayed (2019) reported that in tilapia (*O. niloticus*) the supplementation levels of this microalga (10%, 20% and 30%) resulted proportionally increases of RBC, Hb and Ht values. Diet supplementation of 6 g/kg of *S. platensis* resulted on an increment in hematological parameters and immune enhancement for *O. gouramy*, according to Simanjuntak et al. (2018). Thus, different levels of inclusion of or substitution by *A. platensis* may cause a variety of effects in the immune system of these fish. Hence, it is very important to evaluate the behavior of each species in relation to supplementation provided to them.

The fish innate system includes skin as a physical barrier, the complement system, the antimicrobial enzymes, the interleukins, the interferon, and the organic defense, such as granulocytes, monocytes, macrophages and natural killer cells (Magnadottir et al., 2011). Phagocytosis is the primordial defense mechanism of aquatic organisms and macrophages and plays a pivotal role in the detection of pathogenic microrganisms and in suppress infectious agents (Nickel et al., 1999). Leukocytes are important defense cells and alteration of their normal count on the organism it may suggest the presence of some type of infection (Iftikhar and Hashmi, 2021). Monocytes are the main component of an organism defense and is the largest and the most abundant leukocyte in fish blood (Ranzani-Paiva et al., 2013), besides to act in inflammatory processes and phagocytosis (Hoseinifar et al., 2016). Tambaqui fed with diet supplemented with *A. platensis* (A10) showed reduced numbers of leukocytes, monocytes, eosinophils, and basophils when compared to Control and C10 groups,

indicating an inflammatory process against pathogens (Biller-Takahashi and Urbinati, 2014). Supplementation with *A. platensis* in Nile tilapia (Abdel-Warith and Elsayed, 2019) and in common carp *Cyprinus carpio* (Watanuki et al., 2006) stimulated phagocytosis and superoxide production in leukocytes. In this way, monocytes, eosinophil, and basophil numbers in tambaqui fed with *C. vulgaris*, when compared to those fed with *A. platensis*, could indicate a better immunity of fish, possibly due to immunostimulant components, such as β-1,3-glucan, present in *C. vulgaris* (Raji et al., 2018).

The physiological state of an organism can be assessed analyzing some biochemical metabolites. In this regard, albumin is a protein responsible for nutrient transportation and for regulation of systemic osmotic activity. Increased levels of this protein may indicate an increase in the nonspecific immune response (Guardiola et al., 2018). Dietary *A. platensis* could improve fish immune response due to the presence of phycocyanin and β-carotene in the microalgae composition (Rijal et al., 2020). This improvement occurred in tambaqui that received supplementation of 10% *A. platensis* in comparison with those that only received 5% of the same microalga, indicating that a higher level of supplementation may be beneficial to the fish health. However, tambaqui that received 10% of *A. platensis* presented increased total cholesterol plasma levels. On the other hand, those fish that received both microalgae (*A. platensis* + *C. vulgaris*) in their diet showed reduction on total cholesterol plasma levels that could mean a benefit for *C. macropomum* health. Furthermore, these high cholesterol levels were still lower than those reported for *Dicentrarchus labrax* supplemented with 1% *A. platensis* (Fath El-Bab et al., 2022).

The feed supplemented with *C. vulgaris* and *A. platensis* showed effectiveness against monogeneans present on the tambaqui gills. These microalgae possess both primary bioactive agents and secondary agents such as polyphenols, carotenoids and saponins, among others, which have antifungal, antibacterial and anticancer properties (Bratchkova and Kroumov, 2020; Zielinski et al., 2020). The microalgae *A. platensis* and *C. vulgaris* are rich in phenolic or polyphenolic compounds that act with chemical protection mechanisms against biological agents, ultraviolet radiation, and heavy metals and these products are natural antioxidants and can also protect the organism from free radicals (Alagawany et al., 2021; Andrade et al., 2018; Bratchkova and Kroumov, 2020; Raji et al., 2018). Phenolic acids have substantial lipid and water solubility, and they can be absorbed in the stomach (Rahimi et al., 2022). The microalga C. *vulgaris* presents molecules equivalent to terpenoids, which arisen throughout the evolution of green plants, including defense against herbivores and pathogens, a S-12-hidroxifnolil-L-cistein and this could explain the high effectiveness on tambaqui. Total prevalence of parasitic infestation in marine fish, *Dicentrarchus labrax, Sparus aurata* and *Mugil cephalus* decreased after fed with diets with equal amounts of *Dunula salina*, *Amphora coffeaeformis* and N*annochloropsis*, since these microalgae increased and enhanced the innate immune response in fishes (Ahmed et al., 2020). Thus, it may be presumed that phenolic compounds of microalgae can show high efficacy (up to 70.8%) of the antiparasitic action of dietary *A. platensis* and *C. vulgaris* against monogeneans in juvenile tambaqui.

The microalgae *C. vulgaris* and *A. platensis* supplementation promoted some immunostimulant responses in tambaqui (*C. macropomum*). Higher RBC, monocytes, eosinophil and basophil numbers in tambaqui fed with *C. vulgaris*, when compared to those fed with *A. platensis*, could indicate a better immunity of these fish due to immunostimulant components presence, such as β-1,3-glucan. An advantage for *C. macropomum* health was the reduction on total cholesterol plasma levels in fish that received diet with both microalgae (*A. platensis* + *C. vulgaris*) supplementation compared to the ones that received *A. platensis*. Moreover, there was an efficient reduction in the monogeneans present on the gills of tambaqui fed with diets containing these microalgae *A. platensis* and *C. vulgaris*, in combination or separately, so an antiparasitic action was observed. Nonetheless, further investigations on the supplementation with *A. platensis* and *C. vulgaris*, in combination or separately, besides the quantity of supplementation and duration of feeding should be verified. These additional studies should address the ideal quantities of supplementation with these microalgae in diets for these fish species that are needed for a variety of other benefits to be manifested.

Acknowledgements

The authors thank to the Leandro Fernandes Damasceno for technical support in experimental diet chemical composition analysis. Authors thank their respective universities and institutes for their support. This research was funded by Embrapa, UEAP and UNIFAP.

References

ABDEL-WARITH, A.A.A. and ELSAYED, E.A., 2019 [viewed 13 September 2023]. Use of *Arthrospira platensis* as a feed additive to improve growth performance, feed utilization, body composition, and immune response of Nile tilapia, *Oreochromis niloticus*. *Journal of Scientific and Industrial Research* [online], vol. 78, pp. 681-686. Available from: http://nopr.niscpr.res.in/handle/123456789/50654

AHMED, I.E.N.E.D., EISSA, I.A.M., OSMAN, H.A.M., ZAID, A.A.A. and DARWISH, O.M., 2020. Studies on prevailing parasitic fish diseases in pre-mature cultured Sea bass, *Dicentrarchus labrax*, Sea bream and *Mugil cephalus* at Ismailia, province with special references to control. *International Journal of Veterinary Science*, vol. 9, no. 4, pp. 558-562. http://doi.org/10.37422/IJVS/20.075.

ALAGAWANY, M., TAHA, A.E., NORELDIN, A., EL-TARABILY, K.A. and ABD EL-HACK, M.E., 2021. Nutritional applications of species of *Spirulina* and *Chlorella* in farmed fish: a review. *Aquaculture (Amsterdam, Netherlands)*, vol. 542, pp. 736841. http://doi.org/10.1016/j.aquaculture.2021.736841.

ANDRADE, L.M., ANDRADE, C.J., DIAS, M., NASCIMENTO, C.A.O. and MENDES, M.A., 2018. *Chlorella* and *Spirulina* Microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. *Food Processing & Technology*, vol. 6, no. 1, pp. 45-58. http://doi.org/10.15406/mojfpt.2018.06.00144.

AOAC INTERNATIONAL, 1995. *Official Methods of Analysis of AOAC International*. 16th ed. Arlington, VA: AOAC International.

ARIDE, P.H.R., OLIVEIRA, A.M., FERREIRA, M.S., LIEBL, A.R.S., COMASSETTO, L.E., LADISLAU, D.S., BASSUL, L.A., SILVA, B.R., MATTOS, D.C., LAVANDER, H.D., SOUZA, A.B., POLESE, M.F., RIBEIRO, M.W.S., CASTRO, P.D.S. and OLIVEIRA, A.T., 2021. Growth and hematological responses of tambaqui, *Colossoma macropomum* fed different levels of rice, *Oryza* spp. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 4, pp. 962-968. http://doi.org/10.1590/1519-6984.232560. PMid:33053132.

BILLER-TAKAHASHI, J.D. and URBINATI, E.C., 2014. Fish Immunology. The modification and manipulation of the innate immune system: Brazilian studies. *Anais da Academia Brasileira de Ciências*, vol. 86, no. 3, pp. 1483-1506. http://doi.org/10.1590/0001-3765201420130159. PMid:25211116.

BRATCHKOVA, A. and KROUMOV, A.D., 2020. Microalgae as producers of biologically active compounds with antibacterial, antiviral, antifungal, antialgal, antiprotozoal, antiparasitic and anticancer activity. *Acta Microbiologica Bulgarica*, vol. 36, no. 4, pp. 79-89.

BUSH, A.O., LAFFERTY, K.D., LOTZ, J.M. and SHOSTAK, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, vol. 83, no. 4, pp. 575-583. http://doi.org/10.2307/3284227.

COLLIER, H.B., 1944. Standardization of blood haemoglobin determinations. *Canadian Medical Association Journal*, vol. 50, no. 6, pp. 550-552. PMid:20323122.

CORRÊA, R. O., DE SOUSA, A.R.B. and MARTINS JÚNIOR, H., 2018. *Criação de tambaquis* [online]. Brasília, DF: Embrapa, 20 p. Available from: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/181969/1/Criacao-de-Tambaquis-AINFO.pdf

EIRAS, J.C., TAKEMOTO, R.M. and PAVANELLI, G.C., 2006. *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes*. Maringá: Editora UEM, 199 p.

FAHEEM, M., JAMAL, R., NAZEER, N., KHALIQ, S., HOSEINIFAR, S.H., VAN DOAN, H. and PAOLUCCI, M., 2022. Improving growth, digestive and antioxidant enzymes and immune response of juvenile grass carp (*Ctenopharyngodon idella)* by using dietary *Spirulina platensis. Fishes*, vol. 7, no. 5, pp. 237. http://doi.org/10.3390/fishes7050237.

FATH EL-BAB, A.F., EL-NAWSANY, M.M., EL-BAHLOL, A.A. and ABDEL-KADER, M.F., 2022. The synergy effect of *Spirulina platensis* and/or chitosan nanoparticles on the growth performance, hematological, biochemical parameters, and body composition of the European seabass (*Dicentrarchus labrax*). *Egyptian Journal of Aquatic Biology & Fisheries*, vol. 26, no. 4, pp. 255-271. http://doi.org/10.21608/ejabf.2022.249667.

GOLDENFARB, P.B., BOWYER, F.P., HALL, E. and BROSIOUS, E., 1971. Reproducibility in the hematology laboratory: the microhematocrit determination. *American Journal of Clinical Pathology*, vol. 56, no. 1, pp. 35-39. http://doi.org/10.1093/ajcp/56.1.35. PMid:5556212.

GUARDIOLA, F.A., BAHI, A. and ESTEBAN, M.A., 2018. Effects of dietary administration of fenugreek seeds on metabolic parameters and immune status of gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology*, vol. 74, pp. 372-379. http://doi.org/10.1016/j.fsi.2018.01.010. PMid:29337247.

HOSEINIFAR, S.H., RINGØ, E., MASOULEH, A.S. and ESTEBAN, M.Á., 2016. Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. *Reviews in Aquaculture*, vol. 8, no. 1, pp. 89-102. http://doi.org/10.1111/raq.12082.

IFTIKHAR, N. and HASHMI, I., 2021. Assessment of immunohematological, hematological and biochemical responses in cultivable fish *Cyprinus carpio* exposed to an antibiotic sulfamethoxazole (SMX). *Journal of Water and Health*, vol. 19, no. 1, pp. 108-119. http://doi.org/10.2166/wh.2020.183.

INSTITUTO ADOLFO LUTZ – IAL, 2008. *Métodos físico-químicos para análise de alimentos*. São Paulo: IAL.

ISHIKAWA, N.M., RANZANI-PAIVA, M.J.T. and LOMBARDI, J.V., 2008. Metodologia para quantificação de leucócitos totais em peixe, *Oreochromis niloticus. Archives of Veterinary Science*, vol. 13, no. 1, pp. 54-63. http://doi.org/10.5380/avs.v13i1.11560.

IZEL, A.C.U. and MELO, L.A.S., 2004. *Criação de tambaqui (Colossoma macropomum) em tanques escavados no Estado do Amazonas*. Manaus: Embrapa Amazônia Ocidental, 20 p. Documentos, vol. 32.

MAGNADOTTIR, B., AUDUNSDOTTIR, S.S., BRAGASON, B.T.H., GISLADOTTIR, B., JONSSON, Z.O. and GUDMUNDSDOTTIR, S., 2011. The acute phase response of Atlantic cod (*Gadus morhua*): humoral and cellular responses. *Fish & Shellfish Immunology*, vol. 30, no. 4-5, pp. 1124-1130. http://doi.org/10.1016/j.fsi.2011.02.010. PMid:21338688.

MORADO, C.N., ARAÚJO, F.G. and GOMES, I.D., 2017. The use of biomarkers for assessing effects of pollutant stress on fish species from a tropical river in Southeastern Brazil. *Acta Scientiarum. Biological Sciences*, vol. 39, no. 4, pp. 431-439. http://doi.org/10.4025/actascibiolsci.v39i4.34293.

NICKEL, R., OTT, C., DANDEKAR, T. and LEIPPE, M., 1999. Pore-forming peptides of *Entamoeba dispar*. Similarity and divergence to amoebapores in structure, expression and activity. *European Journal of Biochemistry*, vol. 265, no. 3, pp. 1002-1007. http://doi.org/10.1046/j.1432-1327.1999.00807.x. PMid:10518795.

QUIAO, H., HU, D., MA, J., WANG, X., WU, H. and WANG, J., 2019. Feeding effects of the microalga *Nannochloropsis* sp. on juvenile turbot (*Scophthalmus maximus* L.). *Algal Research*, vol. 41, pp. 101540. http://doi.org/10.1016/j.algal.2019.101540.

RAHIMI, N.N.M.N., NATRAH, I., OH, J.-Y., ERVIN RANZIL, F.K., GINA, M., LIM, S.-H.E., LAI, K.-S. and CHONG, C., 2022. Phytocompounds as an alternative antimicrobial approach in aquaculture. *Antibiotics (Basel, Switzerland)*, vol. 11, no. 4, pp. 469. http://doi.org/10.3390/antibiotics11040469. PMid:35453220.

RAJI, A.A., ALABA, P.A., YUSUF, H., BAKAR, N.H.A., TAUFEK, N.M., MUIN, H., ALIAS, Z., MILOW, P. and RAZAK, S.A., 2018. Fishmeal replacement with *Spirulina platensis* and *Chlorella vulgaris* in African Catfish (*Clarias gariepinus*) diet: effect on antioxidant enzyme activities and haematological parameters. *Research in Veterinary Science*, vol. 119, pp. 67-75. http://doi.org/10.1016/j.rvsc.2018.05.013. PMid:29864632.

RANZANI-PAIVA, M.J.T.R., DE PÁDUA, S.B., TAVARES-DIAS, M. and EGAMI, M.I., 2013. *Métodos para análise hematológica em peixes*. Maringá: EDUEM.

R CORE TEAM, 2020. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

RIBEIRO, F.M., FREITAS, P.V.D.X., DOS SANTOS, E.O., DE SOUSA, R.M., CARVALHO, T.A., DE ALMEIDA, E.M., DOS SANTOS, T.O. and COSTA, A.C., 2016. Alimentação e nutrição de pirapitinga (*Piaractus brachypomums)* e tambaqui (*Colossoma macropomum*): revisão. *Pubvet*, vol. 10, no. 12, pp. 873-882. http://doi.org/10.22256/pubvet.v10n12.873-882.

RIJAL, M.A., SUSANTO, S., SIMANJUNTAK, S.B.I., HERNAYANTI, H. and SUKARDI, P., 2020. Blood Hematological and biochemical parameters of *Osteochilus vittatus* with *Spirulina platensis* supplementation in biofloc system. *Biosaintifika. Journal of Biology & Biology Education*, vol. 12, pp. 431-437. http://doi.org/10.15294/biosaintifika.v12i3.20936.

SILVA, D.J. and QUEIROZ, A.C., 2002. *Análises de alimentos: métodos químicos e biológicos*. 3. ed. Viçosa: Editora UFV, 235 p.

SILVEIRA JÚNIOR, A.M., FAUSTINO, S.M.M. and CUNHA, A.C., 2019. Bioprospection of biocompounds and dietary supplements of microalgae with immunostimulating activity: a comprehensive review. *PeerJ*, vol. 7, pp. e7685. http://doi.org/10.7717/peerj.7685. PMid:31592343.

SIMANJUNTAK, S.B.I., INDARMAWAN, I. and WIBOWO, E.S., 2018. Impact of fed containing different levels of diets supplementation *Spirulina platensis* on growth, haematological, body composition and biochemical parameters, of gurami (*Osphronemus gouramy*). *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 18, no. 5, pp. 681-690. http://doi.org/10.4194/1303-2712-v18_5_04.

TAVARES-DIAS, M., ISHIKAWA, M.M., MARTINS, M.L., SATAKE, F., HISANO, H., DE PÁDUA, S.B., JERÔNIMO, G.T. and DE SÁ, A.R.S., 2009. Hematologia: ferramenta para o monitoramento do estado de saúde de peixes em cultivo. In: A. SARAN NETO, W.S. MARIANO and S.F.P. SÓRIA, orgs. *Tópicos especiais em saúde e criação animal*. São Carlos: Pedro & João Editores, p. 43-80.

VALENTI, W.C., BARROS, H.P., MORAES-VALENTI, P., BUENO, G.W. and CAVALLI, R.O., 2021. Aquaculture in Brazil: past, present and future. *Aquaculture Reports*, vol. 19, pp. 100611. http://doi.org/10.1016/j.aqrep.2021.100611.

WATANUKI, H., OTA, K., TASSAKKA, A.C.M.A.R., KATO, T. and SAKAI, M., 2006. Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. *Aquaculture (Amsterdam, Netherlands)*, vol. 258, no. 1-4, pp. 157-163. http://doi.org/10.1016/j.aquaculture.2006.05.003.

ZAR, J.H., 2010. *Biostatistical analysis*. 5th ed. New Jersey: Prentice-Hall/Pearson, 944 p.

ZHANG, X.P., LI, W.X., AI, T.S., ZOU, H., WU, S.G. and WANG, G.T., 2014. The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea). *Aquaculture (Amsterdam, Netherlands)*, vol. 420-421, pp. 302-307. http://doi.org/10.1016/j.aquaculture.2013.09.022.

ZIELINSKI, D., FRACZYK, J., DEBOWSKI, M., ZIELINSKI, M., KAMINSKI, Z.J., KREGIEL, D., JACOB, C. and KOLESINSKA, B., 2020. B. *Molecules (Basel, Switzerland)*, vol. 25, no. 8, pp. 1790. http://doi.org/10.3390/molecules25081790. PMid:32295155.