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Hemato-biochemical profile of tambaqui (*Colossoma macropomum Cuvier*, 1816) comparing different growth phases in aquaponic systems

Perfil hemato-bioquímico do tambaqui (*Colossoma macropomum Cuvier*, 1816) comparando diferentes fases de crescimento em cultivo aquapônico

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Abstract: The aim of this study was to evaluate the haemato-biochemical parameters of tambaqui *Colossoma macropomum* in different growth phases in an integrated culture with açai *Euterpe oleracea*. For this, 240 juvenile tambaqui with initial average weight and length of 21.8 ± 7.74 g and 11.28 ± 6.88 cm were cultured in an aquaponic system integrated with açai for 180 days. During the period, 108 healthy tambaquis were sampled and categorized into five distinct growth phases. At each growth phase blood aliquots were collected. The first phase being fish with an average weight of 103.1 ± 5.27 g; second phase with 823.4 ± 42.6 g; third phase with 1087.75 ± 16.38 g; fourth phase with 1402.0 ± 76.6 g and fifth phase with 1815.0±65.1 g. Water quality variables remained within acceptable parameters for both cultures. Erythrocyte was significantly lower in the first and second phase. Haemoglobin was significantly lower in fish in the first phase. Haematocrit remained the same from the second phase onwards. MCV was significantly lower in fish with 1815.0 ± 65.1 g. Plasma glucose levels were significantly lower in the first and second phases. Cholesterol, triglycerides, and total proteins were significantly higher in fish of the fifth phase. AST was significantly lower in fish from the third phase when compared to fish from the first and fifth phases. ALT was significantly higher in fish from the first phase when compared to fish from the third, fourth, and fifth phases. The results are important tools for assessing the health and well-being of tambaqui in future research involving aquaponic cultures.

Keywords: Sustainability; Haematology; Amazon; Integrated cultivation; Glucose; Cholesterol; Triglycerides.

Resumo: O objetivo deste estudo foi avaliar os parâmetros hemato-bioquímicos do tambaqui *Colossoma macropomum* em diferentes fases de crescimento em cultivo integrado com açaí *Euterpe oleracea*. Para isso, 240 tambaquis juvenis, com peso e comprimento médio inicial de 21,8 ± 7,74 g e

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11,28 ± 6,88 cm, foram cultivados em sistema aquapônico integrado ao açaí por 180 dias. No período, 107 tambaquis saudáveis foram amostrados e categorizados em cinco fases distintas de crescimento. Em cada fase de crescimento foram coletadas alíquotas de sangue para análises. A 1ª fase avaliou peixes com peso médio de 103,1 ± 5,27 g; a 2ª, peixes com 823,4 ± 42,6 g; a 3ª, peixes com 1.087,75 ± 16,38 g; a 4ª, peixes com 1402,0 ± 76,6 g e a 5ª, peixes com 1815,0 ± 65,1 g. As variáveis de qualidade da água permaneceram dentro dos parâmetros aceitáveis para ambas as culturas. Eritrócitos foram significativamente diminuídos na 1ª e 2ª fase. Hemoglobina foi significativamente diminuída na 1ª fase. O hematócrito manteve-se igual a partir da 2ª fase. O VCM foi significativamente inferior nos peixes com 1815,0 ± 65,1 g. Os níveis de glicose plasmática foram significativamente diminuídos na 1ª e 2ª fases. Colesterol, triglicerídeos e proteínas totais foram significativamente aumentados nos peixes na 5ª fase. AST foi significativamente diminuído nos peixes na 3ª fase, comparado com a 1ª e 5ª fases. ALT foi significativamente aumentado nos peixes na 1ª fase, comparado com a 3ª, 4ª e 5ª fases. Os resultados são ferramentas importantes para avaliar a saúde e o bem-estar do tambaqui em pesquisas futuras envolvendo culturas aquapônicas.

Palavras-chave: Sustentabilidade; Hematologia; Amazonas; Cultivo integrado; Glicose; Colesterol; Triglicerídeos.

1. Introduction

Monocultures have dominated global aquaculture for decades. However, new production methods strive for greater sustainability by integrating fish and vegetables in a model based on the circular bioeconomy known as aquaponics [1], which can sustainably generate food of both animal and plant origin [2]. In Brazil, research on the integrated cultivation of plants with tambaqui (*Colossoma macropomum* Cuvier, 1816) in aquaponic systems has gained prominence in recent years [3, 4, 5].

The tambaqui *C. macropomum* is a species from the Amazon basin [6] and is the leading native fish in Brazilian fish farming, corresponding to 12% of national production, equivalent to approximately 100 thousand tons/year [7]. The tambaqui is also found in Venezuela, Colombia, Peru, and Bolivia and is considered the second largest Amazonian scaly fish, reaching 1 meter in length and approximately 30 kg in weight [6, 8, 9].

Tambaqui can be cultivated using various production modalities [3, 4, 6, 10, 11], with distinct characteristics in each one of them. According to Másílko et al. [12], the culture system can affect the organoleptic properties and lipid composition of the meat of common carp (*Cyprinus Carpio* L.). Stress management, for example, affects the meat quality of Atlantic salmon *Salmo Salar* reared in a nursery [13]. Nevertheless, stocking density did not affect the growth or meat quality of rainbow trout (*Oncorhynchus mykiss* Walbaum) reared in a low-tech aquaponic system [14].

According to Daskalova (15), meat quality reflects the well-being of farmed fish, as they can experience pain and suffering, indicated by metabolic changes. Among several metrics for diagnosing issues in animal welfare, complete blood count stands out.

Hematological analyses can be used to monitor the health status of fish [16]; they can also be performed to quickly and reliably monitor the sanitary conditions of aquaculture,

revealing potential physiological issues, toxicity and biomarkers as well as stress, handling, vaccination, reproduction, and nutritional statuses [17, 18, 19, 20, 21, 22, 23, 24].

Measuring hemato-biochemical parameters in fish blood can show specific patterns and can indicate the health and physiological state of a given species from a specific habitat, according to its age, eating habits, sexual maturation cycle, and stress [25]. Hematological standards have been recently established for several species of cultivated and wild fish [26, 27, 28], however, data is lacking for Brazilian species of commercial interest [29], especially those reared in aquaponics systems. Thus, we herein investigated the haemato-biochemical profile of tambaqui *C. macropomum* in different growth phases in an integrated cultivation with açai *(Euterpe oleracea* Mart, 1824) in aquaponic system.

2. Material and methods

All procedures that involved fish in this study were performed according to ethical principles in animal experimentation and were approved by the Ethics Committee on the Use of Animals (CEUA), protocol number 1457260820.

2.1 Experimental design

A total of 240 juvenile tambaqui *C. macropomum* with an initial average weight and length of 21.8 ± 7.74 g and 11.28 ± 6.88 cm, respectively, were cultured in an aquaponic system integrated with açaí *E. oleracea* for 180 days. During this period, blood samples were collected during different growth phases. The average weights of the fish in the first, second, third, fourth, and fifth phase were 103.1±5.27 g, 823.4±42.6 g, 1087.75±16.38, 1402.0±76.6, and 1815.0±65.1 g, respectively. The experimental units were composed of 12 independent aquaponic systems, in a greenhouse with a rectilinear convective model roof, protected by a shading screen on the sides. Each aquaponic system consisted of a 1,000 L (800 L useful) circular polyethylene tank for fish, with a 70 L decanter, a 100 L biofilter, a pump (3000 L h^{-1}) for water recirculation in the system and a 150 L cultivation bed for açai seedlings (Figure 1 and 2).

The culture environment was evaluated daily by measuring total dissolved solids (TDS) (AQUAREAD AP-800 Multiparameter Probe), electrical conductivity and dissolved oxygen (YSI ProODO, OH, USA, ± 0.01 mg L^{-1}); temperature and pH (BL-1072 - portable digital pHmeter). Ammonia (± 0.03 mg L^{-1}) [30], nitrite (Griess reaction, using APHA [31] methodology, RSD 4%) and nitrate [31], RSD 1.14%), were measured weekly by spectrophotometry (KASUAKI model: IL-593-S) at wavelengths of 630, 540, 220, and 270 nm, respectively. Phosphate levels were measured based on total phosphorus (ascorbic acid) [31].

The fish were fed with extruded commercial feed, offered according to the growth phases: first phase = feed 36% crude protein (CP) and granulometry 3-4 mm, three times daily; second and third phases = feed 32% CP and granulometry 6-8 mm, twice daily; fourth and fifth phases = feed 28% CP and granulometry 8-10mm, twice daily.

Figure 1: Graphical representation of independent aquaponic systems used for integrated culture of tambaqui *C. macropomum* with açaí *E. oleracea* for 180 days. Each aquaponic system consisted of a 1,000 L (800 L useful) circular polyethylene tank for fish, with a 70 L decanter, a 100 L biofilter, a pump (3000 L h–1) for water recirculation in the system and a 150 L cultivation bed for açai seedlings. The figure was designed by the authors using Microsoft® PowerPoint program.

Figure 2: The figure highlights the tambaqui *C. macropomum* after 180 days in aquaponics system weighing approximately 1815 g, and details of the hydroponic bed with seedlings of açai *E. oleracea.*

2.2 Blood sample collection

A total of 108 healthy fish were sampled over the course of the study. Blood was collected from fish with no apparent external signs of disease or physical injury, including lesions on the skin, and pectoral or caudal fins. Samples were collected in five distinct phases during a 180-day fattening cycle. In the first phase, 36 specimens were sampled; in the second, 12 specimens; in the third, 26 specimens; in the fourth, 22 specimens, and in the fifth phase, 12 specimens were sampled.

For collection, the fish fasted for 24 h. Blood samples were collected between 8 and 9 AM. The animals were anesthetized in a solution of Eugenol (50 mg L^{-1}), for approximately 2 minutes. Then they were weighed, measured, and blood was collected by caudal venipuncture [32] using syringes (3.0 mL) with 5% EDTA anticoagulant. The collected blood aliquots were then identified, homogenized, and stored in 2.0-mL Eppendorf tubes at 4°C prior to laboratory analysis. Blood aliquots (approximately 50 µL per sample) were separated for hematological analysis and the rest was centrifuged (KASVI, model: K14-1215), at 1400*g* for 10 min at 4°C to obtain blood plasma for hemato-biochemical analysis.

2.3 Hematological analysis

Erythrocytes were counted in a Neubauer's chamber after dilution 1:200 in Dacie solution. The cyanmethemoglobin technique was used to determine the hemoglobin concentration, using Labtest's commercial kit (reference nº 43-2/10). Hematocrit was determined using the microhematocrit technique [33], where 0.5 µl glass microcapillaries were filled with 3/4 blood and centrifuged in hematocrit microcentrifuge (LOGEN Scientific model: SH-120), at 3000 rpm for 30 min. After centrifugation, the capillaries were read using a microhematocrit card reader scale, with results expressed as percentage. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe [34].

2.4 Hemato-biochemical analysis

Hemato-biochemical analyses were performed using a commercial labtest® diagnostica kits, according to the manufacturer's instructions. Glucose (reference no 133-1/500) was measured using the GOD-Trinder method. Cholesterol (reference no 76-2/100) and triglycerides (reference no 87-2/100) were measured using enzymatic methods. Total proteins (reference no 99-250) were measured using the biuret method. Aspartate aminotransferase (AST) (reference no 109-4/30) and alanine aminotransferase (ALT) (reference nº 108-4/30) activities were measured by kinetic methods in a spectrophotometer (KASUAKI model: IL-593-S) at the wavelength indicated in the kit.

2.5 Statistical analysis

The homoscedasticity and normality of the data were verified. For parametric variables, one-way ANOVA and Tukey's post-hoc tests were used to verify significant differences ($p<0.05$).

For non-parametric results, Kruskal-Wallis, and Dunn's post-hoc tests were used to explore significant differences ($p<0.05$).

3. Results

During the study, the water quality variables in the system showed the following average values: temperature 27.9°C ± 8.7; dissolved oxygen 5.7 ± 1.0 mg L^{-1}; pH 7.0 ± 1.7; ammonia 1.5 ± 1.8 mg L^{-1}; nitrite 0.5 ± 0.6 mg L^{-1}; nitrate 18.5 ± 13.0 mg L^{-1}; phosphate 6.9 ± 0.37 mg L^{-1}; electrical conductivity 340.25 ± 8.30 µS cm^{-1} and TDS 204.6 ± 6.61 mg L^{-1}.

Hematological parameters for erythrocytes, hemoglobin, hematocrit and MCV showed significant differences ($p < 0.05$) between the different growth phases of tambaqui in aquaponics. The numbers of erythrocytes were significantly lower ($p < 0.05$) in the first and second phases, i.e., when the fish weighed between 103.1 ± 5.27 and 823.4 ± 42.6 g; while fish with an average weight of 1815.0 ± 65.1 g (fifth phase) had a higher number of erythrocytes. Hemoglobin was significantly lower ($p < 0.05$) in the blood of fish with an average weight of 103.1 ± 5.27 g (first phase). The hematocrit was the same in fish weighing from 823.4 ± 42.6 g (second phase), however, it was significantly lower ($p < 0.05$) in fish with an average weight of 103.1 ± 5.27 g (first phase). MCV was significantly lower ($p < 0.05$) in fish weighing 1815.0 ± 65.1 g (fifth phase). MCH and MCHC did not show significant differences ($p > 0.05$) between the growth phases (Table 1).

The hemato-biochemical parameters showed significant differences ($p < 0.05$) between the different growth phases of tambaqui. When the fish were smaller, with an average weight between 103.1 ± 5.27 and 823.4 ± 42.6 g (first and second phases), plasma glucose levels were significantly lower ($p < 0.05$) when compared to the other phases. Cholesterol, triglycerides, and total proteins were significantly higher in fish blood with 1815.0 ± 65.1 g (fifth phase). AST were significantly lower ($p < 0.05$) in the blood of fish weighing 1087.75 ± 16.38 g (third phase), when compared to fish from the first and fifth phases. ALT were significantly higher in the blood of fish with an average weight of 103.1 ± 5.27 g (first phase), when compared to fish from the third, fourth, and fifth phases (Table 1).

Table 1: Hemato-biochemical parameters of tambaqui (*Colossoma macropomum*) in different growth phases in an integrated culture with açai *Euterpe oleracea* in aquaponics system. MCV = mean corpuscular volume. MCH = mean corpuscular hemoglobin. MCHC = mean corpuscular hemoglobin concentration. AST = aspartate aminotransferase. ALT = alanine aminotransferase. Data are presented as mean + SD. Different letters are statistically different ($p < 0.05$). (*) Significant.

	Growth phases				
Parameters	*103 g (phase 1)*	*823 g (phase 2)*	*1087 g (phase 3)*	*1402 g (phase 4)*	*1815 g (phase 5)*
*Erythrocytes (× 10^6 µL^{-1})**	1.3±0.3^c	1.5±0.3^c	2.0±0.5^b	2.0±0.4^b	2.4±0.3^a
*Hemoglobin (g dL^{-1})**	5.7±1.8^b	7.6±2.3^{ab}	7.5±2.9^{ab}	9.6±1.6^a	9.9±1.3^a
*Hematocrit (%)**	26.01±4.4^b	32.9±7.82^{ab}	40.7±11.6^a	38.0±5.0^a	37.4±3.8^a
*MCV (fL)**	207.4±81.2^a	221.8±69.1^a	211.8±59.9^a	210.0±45.5^a	158.2±19.9^b

MCH (pg)	48.9±18.8	53.7±16.9	40.4±19.6	52.9±13.9	41.9±6.1
MCHC (g dL^{-1})	23.9±10.3	26.1±6.6	20.5±10.4	26.3±5.3	26.6±2.7
*Glucose (mg dL^{-1})**	44.9±10.6^{b}	57.7±12.6^{b}	86.7±17.9^{a}	87.6±30.6^{a}	88.0±10.3^{a}
*Cholesterol (mg dL^{-1})**	66.9±20.45^{c}	117.2±15.2^{ab}	113.9±23.9^{b}	118.8±26.4^{b}	229±86.6^{a}
*Triglycerides (mg dL^{-1})**	210.2±79.0^{d}	225.7±52.4^{cd}	307.2±71.4^{bc}	341.9±67.7^{ab}	602.7±357.3^{a}
*Total Proteins (g dL^{-1})**	3.2±0.53^{b}	2.6±0.24^{c}	2.9±0.5^{bc}	2.57±0.5^{c}	4.42±0.3^{a}
*AST (UL^{-1})**	97.5±41.7^{a}	75.7±17.5^{ab}	54.84±10.6^{b}	73.36±20.7^{ab}	83.7±21.5^{a}
*ALT (UL^{-1})**	60.4±31.4^{a}	32.3±17.8^{ab}	19.2±5.3^{b}	20.6±13.8^{b}	23.2±5.9^{b}

4. Discussion

Hematological analyses are commonly performed to assess fish health and welfare in aquaculture research [16]. Hematological parameters are highly sensitive to environmental factors including nutrition, water quality, stress, and pathogens [35]. In the present study, we measured several hemato-biochemical parameters in tambaqui *C. macropomum* across growth phases in an integrated culture with açai *E. oleracea* in an aquaponics system, which can support and guide future investigations. Notably, the data were obtained in EDTA-containing plasma, which may differ from studies that measure serum biochemistry.

In addition to the type of farming system, water quality parameters can affect the fat content and fatty acid profile of fish [12], highlighting the importance of production systems in the final quality of fish. In aquaponic sets, like the model presented herein, plants can directly interfere with the amount of nitrogenous and phosphate compounds available in the water [5], reducing the concentrations of ammonia, nitrite, nitrate, and orthophosphates, thereby improving fish health and quality.

In aquaponic systems, water quality is essential for the performance and well-being of both animals and plants as well as production [36]. In this study, the water quality variables temperature, dissolved oxygen, pH, ammonia, nitrite, nitrate, phosphate, electrical conductivity, and TDS remained within acceptable limits for the development of both cultures [4, 37]. However, constant monitoring is essential, because water quality can directly affect the hematological profile of fish [38].

Svetina et al. [39] revealed a marked seasonal and age-dependent variation in the hemato-biochemical variables of the blood of carp *C. carpio* kept in small ponds with water quality under good environmental conditions. The plasma glucose concentration of carp increased by 50% in the third year, accompanied by an even greater increase (80%) in the total lipid concentration. Despite this, no considerable changes in cholesterol and total protein concentrations were observed. These hemato-biochemical variables could be used to monitor the metabolic balance and health status of intensely cultivated fish. Likewise, the data obtained in the present study will serve as a library to assess the health status of tambaqui cultivated under conditions similar to those described here.

The hematological parameters differed across growth phases. The total erythrocyte count increased as the tambaqui size increased. Fazio et al. [40], Adeyemo et al. [25], Svetina et al. [39],

Ikechukwu and Obinnava [41] and Arnaudov et al. [42], also observed increased erythropoiesis during fish growth and especially during the breeding season.

Similarly, hemoglobin content increased with the size of the fish. This should be expected, as the amount of hemoglobin during homeostasis correlates with the number of circulating erythrocytes. As observed in other studies, the function of hemoglobin adapt to metabolic and environmental changes. The hematocrit value depends on the number and size of erythrocytes and can be affected by several factors such as body weight, as observed in this study [43]. Several immature erythrocytes in tambaquis that weighed 1815.0±65.1 g (fifth phase) would also justify a lower MCV in this same group.

Higher MCV values in the early stages of fish life may be related to greater cell production [44]. As fish grow, these immature cells differentiate, decreasing the nuclear-cytoplasm ratio and condensing chromatin, which therefore decreases cell size and MCV [43].

Although Costa et al. [45] measured different values for the hematological parameters of juvenile tambaqui *C. macropomum* (±70 g), such differences may be related to stress, as the animals were subjected to different stocking densities in concrete tanks. On the other hand, Dias et al. [46] found similar values to those reported in the present study for erythrocytes and hematocrit in juvenile tambaqui (final average weight 32.4 ± 0.8 g) cultured in a clear-water recirculation aquaculture system, indicating patterns in the results when culture systems have similarities.

Hemato-biochemical parameters can reveal stressful physiological conditions in tambaqui [47]. Glucose is the main source of energy for many organic functions, and blood levels vary according to the size, metabolic requirements, and stress of the animal [48, 49, 50]. The plasma cholesterol content found in most teleost fish is approximately two to six times higher than that in mammals. Hypercholesterolemia, though physiologically common in many teleosts and not apparently associated with disease, is influenced by factors such as age, growth, gender, diet, and nutrition [51]. Fat storage in tambaqui may be related to gametogenesis [52], as shown by Vieira [53] for curimbatá (*Prochilodus scrofa* Steindachner, 1881), in which the highest levels of blood lipids were measured in the maturation phase (i.e., during intense lipid mobilization for vitellogenesis and spermatogenesis), which could explain the findings of the present study.

In general, total plasma proteins constitute a very unstable biochemical system, reflecting the condition of the organism and the changes that occur under the influence of autogenous and exogenous factors [54]. The plasmatic protein observed in the first phase may be related to the diet that contained a higher percentage of crude protein, considering that increased plasma protein due to increased protein levels in the fish diet has also been observed by Abdel-Tawwab [55] and Abdel-Tawwab et al. [56]. On the other hand, the plasmatic protein observed in the fifth phase may be related to sexual maturation, a protein-driven process [57].

Oliveira and Val [20] explored how various climatic scenarios affect the growth and physiology of tambaqui. They found that climate changes affect physiology and hemato-biochemical parameters, such as blood glucose, cholesterol, and plasma triglycerides. In

addition, it was found that tambaqui can recover blood parameters to baseline, suggesting an artificial acclimatization to adverse environmental conditions. In the present study, the fish were not subjected to stressful conditions, nor were they fed with enriched diets that could eventually alter the haemato-biochemical parameters. However, the fish in the control group of Oliveira and Val [20] showed similar results to those in the present study, indicating that aquaponic systems offer good cultivation conditions for tambaqui.

ALT) and AST activities in the blood plasma of African catfish (*Clarias gariepinus* Burchell, 1822) increased significantly after exposure to potassium permanganate, and were used as stress indicators [58]. AST and ALT are present in liver cells and are released into the blood following liver damage, thus rendering them useful markers for diagnosing and monitoring liver diseases. However, both are transaminases, i.e., enzymes that can be measured in the blood to reflect the functional status of the liver [59]. According to Chen et al. [60], the liver of fish from aquaculture may present abnormalities due to nutritional imbalances in the formulation of commercial diets. On the other hand, Zachary et al. [61] found that the liver is responsible for the metabolic degradation of triglycerides. This may explain the high metabolic activity measured in the tambaqui liver in this study, indicating healthy functioning of the organ.

Some hemato-biochemical parameters are sensitive to environmental fluctuations and indicate physiological disturbances before the onset of external symptoms; therefore, it is necessary to reduce the stress of the fish as much as possible [35]. In recent decades, the welfare of fish during all phases of cultivation has been prioritized both for ethical and commercial reasons, striving for meat quality [15].

In this context, aquaponics proves to be an effective and sustainable tool, as it enables the integrated production of fish with vegetables in a closed system while saving water and recycling nutrients. This ensures production cycles year-round and the welfare of the tambaqui.

5. Conclusion

This study measured several haemato-biochemical parameters during several growth phases in tambaqui *C. macropomum* in an integrated culture with açai *E. oleracea* in an aquaponics system. Our data revealed differences in these parameters across growth phases; they may also vary across species and types of culture. This study will guide future work on evaluating the health and functionality of tambaqui in aquaponic cultures.

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Conflict of interest

The authors declare there are no competing interests.

Author contributions

Paola Fabiana Fazzi-Gomes: Methodology, investigation, data curation, writing – original draft. Helen Cristiane Araújo Souza: Investigation, methodology. Marcela Cardoso Sena: Investigation, data curation. Joane Natividade

Souza: Methodology, investigation. Marco Shizuo Owatari: writing – original draft, writing—review and editing. Fabio Carneiro Sterzelecki: Funding acquisition, conceptualization, methodology. Nuno Filipe Alves Correia Melo: Funding acquisition, conceptualization. Glauber David Almeida Palheta: Funding acquisition, conceptualization, methodology, validation, supervision.

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