ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392202404182

ANIMAL AND FISHERIES SCIENCES | ORIGINAL ARTICLE

Aquaculture feeds produced with Amazon by-products in accordance with organic certification standards: effect on growth and health of *Colossoma macropomum*

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ABSTRACT

This study evaluated the effects of organic diets formulated with Amazonian by-products on the productive parameters and health of juvenile tambaqui (Colossoma macropomum). Four experimental diets were tested: CL (Cassava Manihot esculenta leaf meal), BN (defatted Brazil Bertholletia excelsa nut meal), CLBN (a combination of CL and BN), and FFBN (fish residue meal, black soldier fly larvae Hermetia illucens meal, and BN). A total of 180 fish (14.37 ± 0.50 g) were distributed across 12 tanks (400 L) and fed for 60 days in a semi-closed system. Growth performance, body composition, metabolic, and immune parameters were assessed. Fish fed the BN diet exhibited superior weight gain (+35.34%), specific growth rate (+15.73%), and protein (+20.80%) and energy (+23.02%) retention compared to the CL diet ($p < 0.05$). The inclusion of 400 g/kg of cassava leaf meal reduced intestinal trypsin activity by 66%, negatively impacting feed conversion efficiency (CL: 1.41 vs. BN: 1.18). While the FFBN diet supported growth, it increased visceral fat and triglyceride levels. The CLBN diet negatively affected the immune response. Results suggest that the BN diet is the most suitable for organic production, promoting better growth and economic return without altering metabolic conditions, body composition, or tambaqui health. The use of Amazonian by-products in organic feed presents a sustainable alternative, fostering the integration of family-based aquaculture into the circular bioeconomy of the Amazon region.

KEYWORDS: Brazil nut, cassava leaf, enzymatic activity, immune response, welfare

Rações para aquicultura produzidas com subprodutos da Amazônia de acordo com os padrões de certificação orgânica: efeito no crescimento e na saúde de *Colossoma macropomum*

RESUMO

O estudo avaliou o impacto de dietas orgânicas formuladas com subprodutos da Amazônia nos parâmetros produtivos e saúde de juvenis de tambaqui (Colossoma macropomum). Quatro dietas experimentais foram testadas: CL (Farinha de folha de mandioca Manihot esculenta), BN (Torta de Castanha do Brasil Bertholletia excelsa desengordurada), CLBN (combinação de CL e BN) e FFBN (resíduos de pescado, larvas de mosca soldado negro Hermetia illucens e BN). Os 180 peixes (14,37 ± 0,50 g) foram distribuídos em 12 tanques (400 L) e alimentados por 60 dias em um sistema semifechado. O desempenho zootécnico, composição corporal, parâmetros metabólicos e imunológicos foram avaliados. Os peixes alimentados com a dieta BN apresentaram maior ganho de peso (+35,34%), taxa específica de crescimento (+15,73%) e retenção de proteína (+20,80%) e energia (+23,02%) em comparação à dieta CL ($p < 0,05$). A inclusão de 400 g/kg de farinha de folha de mandioca reduziu em 66% a atividade de tripsina intestinal, comprometendo o desempenho e a conversão alimentar (CL: 1,41 vs. BN: 1,18). Embora a dieta FFBN tenha favorecido o crescimento, aumentou os níveis de gordura visceral e triglicerídeos, enquanto a CLBN impactou negativamente na resposta imune. Os resultados indicam que a dieta BN é mais adequada para a produção orgânica, promovendo maior crescimento e retorno econômico sem alterar o metabolismo, composição corporal ou saúde do tambaqui. O uso de subprodutos amazônicos para ração orgânica oferece uma alternativa sustentável, integrando aquicultura familiar à bioeconomia circular na Amazônia.

PALAVRAS-CHAVE: castanha do Brasil, folha de mandioca, atividade enzimática, resposta imune, bem-estar

CITE AS: Medeiros, P.A.; dos Santos, R.B.; Matos Dantas, F.; Monteiro dos Santos, D.K.; Freitas, T.M.; Gonçalves, L.U.; Affonso, E.G. 2025. Aquaculture feeds produced with Amazon by-products in accordance with organic certification standards: effect on productive parameters and health of *Colossoma macropomum*. *Acta Amazonica* 55: e55af24418.

INTRODUCTION

In 2020, the global organic market reached approximately 121 billion euros, with Europe and North America leading in consumption (Schlatter *et al*. 2022). The expansion of this sector is driven by the growing demand for more sustainable food and production practices, as well as increasing concerns over pesticide use and the environmental impact of conventional agriculture (Hyland *et al*. 2019; Fagan *et al*. 2020). In aquaculture, organic production still represents a small share of global output. However, it has demonstrated strong growth potential, primarily due to the rising preference for production systems and inputs that prioritize animal welfare and the conservation of aquatic ecosystems (Beg *et al*. 2024).

One of the key challenges in advancing organic aquaculture is the development of diets that comply with the specific legislative requirements of each region. This requires comprehensive research on nutrition and the effects of organic diets on fish health and meat quality (Mente *et al*. 2011; Gambelli *et al*. 2019; Sicuro 2019). Most studies on organic aquafeeds focus on the partial replacement of fish by-products with more sustainable organic protein sources (Lund *et al*. 2011). Organic ingredients – such as insects, plant-based by-products, and animal-derived residues – hold significant potential as viable alternatives to completely replace high-environmental-impact diets in aquaculture, with promising economic returns when compared to fishmeal-based diets (Tefal *et al*. 2024).

In organic aquaculture, it is preferable to farm species that feed on low-trophic-level organisms (Mente *et al*. 2019). Tambaqui (*Colossoma macropomum* Cuvier 1818) possesses several characteristics that make it well-suited for organic farming, including its ability to utilize phytoplankton and zooplankton as food sources (Arantes and Freitas 2016), its resilience to higher stocking densities without compromising welfare parameters across different production systems (Izél-Silva *et al*. 2020; Dos Santos *et al*. 2023), and its high efficiency in digesting and absorbing nutrients from plant-based ingredients (Guimarães *et al*. 2014; Nascimento *et al*. 2020).

Despite being the most farmed native freshwater fish species in Latin America and cultivated in other regions such as Central America and Asia (Hilsdorf *et al*. 2021), there is still limited information on the use of organic diets for tambaqui. The utilization of high-value biological byproducts from the Amazon, predominantly produced in organic systems, can serve as an alternative for the cultivation of tambaqui, the region's primary aquaculture species (Medeiros *et al*. 2018; Matos-Dantas *et al*. 2024).

The Amazon region hosts the largest tropical forest on the planet, and for socio-environmental reasons, local production of feed inputs is limited compared to other regions in the southern part of the continent. Additionally, around 90% of aquaculture production in the state of Amazonas occurs in small-scale systems (Lima *et al*. 2020). The socio-economic aspects of organic aquaculture are particularly relevant for developing regions, as this practice can contribute to improved livelihoods and can be effectively integrated with agricultural activities and the local circular bioeconomy (Gambelli *et al*. 2019). Thus, this study aimed to evaluate the effects of four diets formulated with Amazonian ingredients, in accordance with organic certification standards, on the growth, body composition, metabolic parameters, and welfare of tambaqui in aquaculture systems.

The ingredients for the diets were chosen based on their nutritional composition, production volume and low utilization in the region. Protein composition was a criterion for selecting cassava (*Manihot esculenta* Crantz) leaf meal, defatted Brazil nut cake (*Bertholletia excelsa* Humb. and Bonpl), fish waste meal and black soldier fly (*Hermetia illucens* Linnaeus 1758) larvae meal. Guarana bagasse (*Paullinia cupana* Kunth var. sorbilis) and cassava starch were used as energy sources and to promote the agglutination of the feed granules. Despite the potential of the ingredients, the evaluation of the effect of the mixtures between the ingredients on the productive and health indicators of the animals is essential for their application in family aquaculture systems.

MATERIAL AND METHODS

Ethics authorization and experimental design

The experiments were carried out at the Aquaculture Experimental Station of the Instituto Nacional de Pesquisas da Amazônia (INPA) (3º 05' 24" S 59º 59' 36" W) under authorization from the INPA's Ethics Committee for the Use of Animals in Research (No. 020/2020, SEI 01280.000410/2020-76).

The experimental design was completely randomized, four treatments were tested in triplicate (n=3), which were: Diet based on Cassava leaf meal (CL), Diet based on defatted Brazil nut meal (BN), Diet based on a combination of cassava leaf meal and defatted Brazil nut meal (CLBN), and Diet based on fish residue meal, black soldier fly larvae meal, and defatted Brazil nut meal (FFBN).

Origin and processing of ingredients

Guarana (*Paullinia cupana* Kunth var. sorbilis) bagasse, leaves and cassava starch were purchased from certified producer's participants in the participatory guarantee system Rede Maniva de Agroecologia, Manaus, Brazil. The leaves and the upper third of the plant stem were ground and dried at 50ºC for 72 hours to produce cassava leaf meal. The cassava starch was obtained from the decantation of the residual liquid from pressing the crushed root. Guaraná bagasse was obtained by dehydration of the seeds (<12% moisture), followed by crushing (3 – 5 mm) and hydroalcoholic extraction of guaranine. Fish waste meal was produced from filleting waste and whole fish unfit for human consumption, in a 1:1 ratio, ground, cooked at 100 °C for 10 minutes and dried for 120 minutes at 70 °C in a 1.5 m diameter iron furnace. Defatted Brazil nut meal came from organic extractivism. Seeds that did not meet human consumption standards were pressed at 12 atm of pressure to extract the oil, the residual cake was dried at 50ºC for later use in feed. Black soldier fly larvae were produced in an organic substrate without synthetic additives. The raw materials of the non-organic ingredients (poultry by-product meal, meat and bone meal and swine fat) were obtained from free-range animals kept in a feeding system with native pasture. The composition of the diets and the origin of the ingredients are described in Table 1.

Analysis of the centesimal and amino acid composition

The centesimal composition of ingredients, feeds and whole-body fish were analyzed following the rules of the Association of Official Analytical Chemists (AOAC 2005). The gross energy was calculated by incinerating the samples using a bomb calorimeter. Amino acid analysis was performed using high performance liquid chromatography (Figure 1) (White *et al*. 1986).

Table 1. Formulation and proximal composition of experimental organic diets.

Ingredients	Diets[1]			
	CL	BN	CLBN	FFBN
Organic ingredients[2]				
Fish residue meal	132.10	125.00	120.00	250.00
Black soldier fly meal	120.00	100.00	114.00	210.00
Cassava leaf meal	400.00	0.00	200.00	130.00
Defatted Brazil nut meal	0.00	400.00	200.00	225.00
Guarana bagasse	68.00	75.10	76.10	85.00
Cassava starch	80.00	100.00	90.00	100.00
Non-organic ingredients[3]				
Poultry by-product meal	160.00	160.00	160.00	0.00
Meat and bone meal	0.00	39.90	19.90	0.00
Swine fat	39.90	0.00	20.00	0.00
Proximal composition (g kg^{-1})[1]				
Dry matter	913.37	923.40	916.70	891.77
Crude protein	320.40	329.40	319.60	328.50
Digestible protein[4]	289.02	289.98	287.01	285.41
Lipids	56.78	61.92	63.94	60.56
Ash	94.65	96.92	100.91	105.00
Crude fiber	77.32	79.78	74.70	78.95
Acid detergent fiber	94.43	94.11	95.60	85.53
Neutral detergent fiber	207.60	200.90	215.70	190.00
Lignin	3.17	2.03	2.89	2.12
Cellulose	62.73	73.82	66.70	64.33
Hemicellulose	113.17	106.79	120.10	104.47
Feed cost (US$)[4]	0.79	0.68	0.73	0.75
Digestible energy (MJ Kg^{-1})[5]	16.46	16.09	16.29	16.67

[1]CL: diet based on cassava leaf meal. BN: diet based on defatted Brazil nut meal. CLBN: diet based on cassava leaf meal and Brazil nut meal. FFBN: fish residue, black soldier fly larvae and defatted Brazil nut meals.
[2]Organic ingredients: Fish residue meal obtained from Frigorífico Maués©; black soldier fly larvae meal obtained from Natuprotein©; Cassava leal and starch obtained from Association of Rural Farmers of the São Raimundo do Mutuca Community© and Boa Vista do Janauacá Community ©; Defatted Brazil nut meal obtained from Amazon Oil©; Guarana bagasse produced by Agricultural Community Association of the Urupadi River and processed by AMBEV Maués©.
[3]Ingredients from the Maués cold store©.
[4]Feed cost calculated in reais and converted for dollars (R$ 1.00 = US$ 5.74).
[5]Protein and digestible energy calculated based on a previous digestibility test (Medeiros, 2022) and on the results of Guimarães ***et al***. (2014), Buzollo ***et al***. (2018) and Monteiro dos Santos ***et al***. (2022).

Experimental feeds

The four isoproteic and isoenergetic diets were balanced according to the recommendations of Buzollo *et al*. (2019) and were produced free of transgenic ingredients or derivatives and synthetic additives. The CL, BN and CLBN diets were prepared following the Brazilian normative instruction No. 28 (MAPA and MPA 2011), which establishes technical standards for organic aquaculture production systems in Brazil. According to this normative instruction, the use of non-organic foods in proportion to the daily intake of up to 20% based on dry matter is allowed. The diet FFBN was balanced according to the European Union (EC 2007) and International Federation of Organic Agriculture Movements standards (IFOAM 2014).

All ingredients were dried by heating (< 70 ºC), then subsequently ground to 1 mm granulometry in a hammer mill. The diets were processed in a single screw extruder machine (MX-80, Inbramaq, SP, BR) with a 4 mm die and then dried in an oven with circulation and air renewal at 60 ºC for 8 h.

Feeding trial

The feeding trial was carried out using 180 tambaqui (14.37 ± 0.50 g) purchased from Santo Antônio Farm, Rio Preto da Eva, Amazonas, Brazil (2° 44' 41.5" S 59° 28' 48.7" W). The fish were distributed in 12 tanks of 400 L useful volume (15 fish per tank; n = 3 per treatment) and fed three times a day (09:00, 13:00 and 17:00) with experimental diets until apparent satiation, for 60 days.

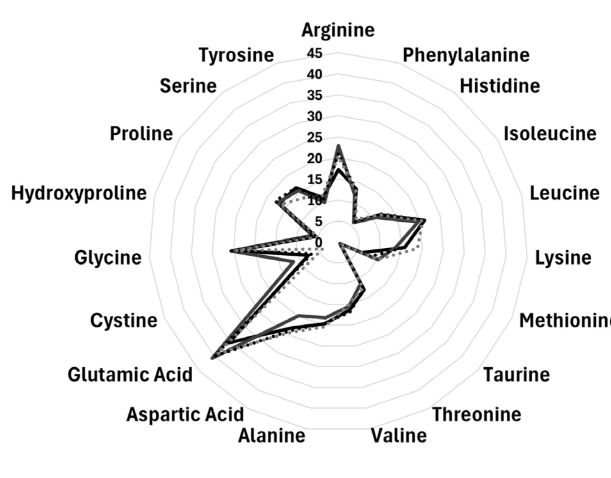

Figure 1. Amino acid composition of experimental diets: CL: diet based on cassava leaf meal. BN: diet based on defatted Brazil nut meal. CLBN: diet based on cassava leaf meal and Brazil nut meal. FFBN: fish residue, black soldier fly larvae and defatted Brazil nut meals.

The tanks were equipped with an aeration system, and the photoperiod was set to alternate between 12 hours of darkness and 12 hours of light. Water quality was maintained using a semi-static system with partial tank volume renewal. Every three days, the sedimented solids were removed and 75% of the water volume in each tank was renewed. The volume was completed with water from an artesian well and corrected to pH 7 using sodium bicarbonate.

The values (mean ± standard error) of temperature (27.79 ± 0.28 °C), dissolved oxygen (5.85 ± 0.05 mg L^{-1}), pH (6.84 ± 0.30 H^+), salinity (0.01 ± 0.00 mg L^{-1}) and electrical conductivity (89.48 ± 2.94 mS cm^{-1}) were measured daily with a multiparameter probe. Total ammonia (1.64 ± 0.30 mg L^{-1}) and toxic ammonia (0.00 ± 0.00 mg L^{-1}) were analyzed according to Verdouw *et al*. (1978). The values of nitrite (0.38 ± 0.09 mg L^{-1}), total alkalinity (18.43 ± 1.75 $CaCO_3$ mg L^{-1}) and total hardness (10.99 ± 0.84 Ca^{+2} + Mg^{+2} mg L^{-1}), were measured once a week according to the Boyd and Tucker (1992). The water quality parameters were within the limits considered comfortable for tambaqui rearing (Wood *et al*. 2017; Barroso *et al*. 2020).

Growth performance, somatic indices, feed cost, and whole-body composition

At the end of the experimental period, all fish were anesthetized with benzocaine (50 mg L^{-1}) (Gomes *et al*. 2007), measured, and weighed. With the biometric data, body composition and consumption of the diets by the fish, the following metrics were calculated: Survival rate (S %) = 100 × (Final fish number / Initial fish number); initial weight (IW g) or final weight (FW g) = Σ fish initial or final weight / number of fish, Weight Gain (WG %) = [(FW - IW) / IW] x 100; Daily Weight Gain (g day^{-1}) = average weight gain (g) / number of days; Daily Feed Intake (DFI % live weight day^{-1}) = [(feed intake / number of days) / (IW + (WG / 2)] x 100; Feed Conversion Ratio (FCR) = Feed intake (g) / WG (g); , Specific Growth Rate (SGR % live weight day^{-1}) = [(ln FW – ln IW) / number of days] x 100, Protein Retention (%) = [(FW x final body protein) – (IW x initial corporal protein) / protein ingestion] x 100; and Energy Retention (%) = [(FW x final body energy) – (IW x initial corporal energy) / energy ingestion] x 100. Calculations were performed according to NRC (2011).

The cost of ingredients and feeds was calculated by adding the selling price of suppliers, transportation and processing costs. The manufacturing cost was calculated from the sum of expenses with labor, electricity, cost amortization and depreciation of equipment per kg of feed produced. The average cost of commercial feed was calculated based on price consultations with six feed suppliers in the Amazon region in April 2025. The feed cost per kg of live weight produced was calculated according to Wang *et al*. (2021).

For the analysis of the somatic indices and body composition, six fish were anesthetized and euthanized before the experiment for collecting initial body composition. At the end of the experimental period, six fish per treatment (n = 6) were anesthetized and euthanized at the end of the experimental period, in both cases with a high dose of benzocaine (> 500 mg L^{-1}), followed by spinal medulla rupture, according to the rules of the CONCEA (2018). Via the weight of the organs, the following were calculated: Viscerosomatic Index (VSI %) = (visceral weight / fish weight) × 100; Hepatosomatic Index (HSI %) = (liver weight / fish weight) × 100; Visceral Fat Index (VFI %) = (fat weight / fish weight) × 100; and Intestine length/total length ratio. Body composition analyses were performed according to item 2.3.

Blood parameters

At the end of the experiment, nine fish per treatment (n = 9) were anesthetized with benzocaine (50 mg L^{-1}) (Gomes *et al*. 2007) and blood was collected by puncturing the caudal vessel. Blood samples were divided into vials with and without an anticoagulant solution of ethylenediaminetetraacetic acid (EDTA 10%) (Sousa *et al*. 2021).

From blood, the following items were analyzed: number of erythrocytes - RBC (erythrocytes 10^6 μL^{-1}) using a formaldehyde citrate solution in a hemocytometer; hematocrit - Ht (%) using the microhematocrit method; hemoglobin concentration - [Hb] (g dL^{-1}) using the cyanmethemoglobin method. Hematimetric indices were calculated following the methodology of Wintrobe (1934).

From the serum, cortisol (ng ml^{-1}) was analyzed using ELISA method, and total proteins (g dL^{-1}) were analyzed using the biuret method (method 99, Labtest, MG, BR).

Blood plasma was analyzed for glucose (mg dL^{-1}) (method 33, Labtest, MG, BR); cholesterol (mg dL^{-1}) (method 76 Labtest method, MG, BR); triglycerides (mg dL^{-1}) (method 87, Labtest, MG, BR); alanine amino transferase - ALT (U L^{-1}) (method 1008, Labtest, MG, BR) and aspartate amino transferase - AST (U L^{-1}) (method 109, Labtest, MG, BR) levels.

Innate immune response

The leukocyte respiratory activity (LRA) was measured using the reduction reaction of nitroblue tetrazolium with reading in a spectrophotometer at 545 nm, as described by Siwicki *et al*. (1994). Blood smears were stained with May Grünwald-Giemsa-Wright dye and the total number of thrombocytes and leukocytes were quantified, as described by Gonzales *et al*. (2020).

Digestive enzyme activity

At the end of the experiment, nine fish per treatment (n = 9) were euthanized with a lethal dose of benzocaine (500 mg L^{-1}). For stomach acid protease analyses, the enzymatic extract was prepared by diluting (1:1) the contents of the stomach of the fish with distilled water. The enzymatic extract of the intestine was prepared using a similar process, with dilution of the sample in distilled water at 1:5. The protein concentration of the homogenates was determined according to Bradford (1976).

The activity of acid proteases was determined according to Anson (1938). Trypsin activity was determined using the method described by Erlanger *et al*. (1961). The α-amylase activity according to the method described by Bernfeld (1955), with adaptations. The reaction was initiated by the addition of starch to the reaction medium and stopped after 30 min with the addition of 1 mL of solution containing 1% 3,5-dinitrosalysilic acid, 8% NaOH and 30% double sodium and potassium tartrate, heated in a boiling water bath for 6 min, cooled and diluted in 10 mL of distilled water. Lipase activity was continuously determined according to the methodology of Winkler and Stuckman (1979).

Statistical analysis

Data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). The comparison of treatments was performed using one-way ANOVA and means were compared using Tukey's test. All analyses were performed at a significance level of 5% using the statistical software Statistica (version 7.1).

RESULTS

The survival rate was 100% and the fish did not exhibit any clinical signs or refused the pellets. Despite having the same daily feed intake and similar growth performance indicators as the fish fed FFBN, the fish from the CL treatment had significantly lower ($p < 0.05$) weight gain compared to the CLBN. Additionally, FCR, SGR, protein, and energy retention were significantly ($p < 0.05$) worsened in fish from the CL treatment compared to fish from the BN. The feed cost per kg of live weight produced was significantly ($p < 0.01$) higher for the CL diet compared to the others. The values ranged from US$ 0.81 to US$ 1.11 per kg of live weight produced (Table 2). The average cost of extruded commercial feed was US$0.72.

The visceral fat index showed a significant increase ($p < 0.05$) in the fish fed with the FFBN diet compared to those fed CL and CLBN diets. Moisture content in bodies of the fish was significantly higher ($p = 0.01$) in those fed with CLBN compared to those fed with BN (Table 3). Fish fed the FFBN diet had significantly higher values of cortisol ($p = 0.025$) compared to those fed the BN diet, and higher triglycerides ($p < 0.001$) were observed compared to the groups of fish fed the other diets (Table 4).

A significant increase ($p = 0.013$) was observed in the leukocyte count of fish fed the CL diet in relation to the CLBN (Table 4). Total lymphocytes were higher in fish ($p = 0.017$) fed the FFBN diet compared to the CLBN diet. Eosinophils and specific granulocytic cells were not found. Fish fed the FFBN diet showed higher trypsin activity compared to fish fed the CL diet (Table 5). There was influence of the diets on amylase activity ($p = 0.040$), in which fish fed with CLBN and FFBN showed values of around 30% greater than those fed with CL and BN.

DISCUSSION

Organic aquaculture is a holistic approach to farm management and food production that combines best environmental practices, maintains biodiversity, conserves natural resources, and guarantees animal welfare (Mente *et al*. 2019). In this study, the use of under exploited organic raw materials in agro-industries of the Amazon to produce organic aquaculture feed was a technically and economically viable alternative to traditional commercial feeds, especially considering the low availability of ingredients for processing aquaculture feed in the region. The growth and weight gain of the fish were within the range observed in other studies involving the rearing of tambaqui with non-organic diets (Buzollo *et al*. 2019; Izél-Silva *et al*. 2020). The protein retention of the tambaqui (38.5 – 46.5%) was close to the values found for tilapia fed high-efficiency diets supplemented with synthetic amino acids, proteases, and a mixture of organic acids (Huan *et al*. 2019). Tambaqui fed diets based on traditional ingredients, with

Table 2. Growth performance (mean ± standard error) of *Colossoma macropomum* fed experimental organic diets. DWG: Daily weight gain, DFI: Daily feed intake, FCR: Feed conversion ratio, SGR: Specific growth rate.

Parameters	Diets[1]				P-value[2]
	CL	BN	CLBN	FFBN	
Initial weight (g)	14.51±0.15	14.22±0.40	14.15±0.41	14.60±0.22	0.701
Final weight (g)	72.33±5.47^{b}	94.12±0.56^{a}	85.84±2.65^{ab}	89.36±5.21^{ab}	0.025
Weight gain (%)	399.04±40.95^{b}	540.05±6.85^{a}	528.19±14.90^{a}	511.40±27.75^{ab}	0.018
DWG (g dia^{-1})	0.96±0.09^{b}	1.32±0.01^{a}	1.20±0.04^{ab}	1.25±0.08^{ab}	0.024
DFI (% dia^{-1})	2.44±0.00	2.27±0.08	2.38±0.04	2.23±0.07	0.122
FCR	1.41±0.05^{a}	1.18±0.04^{b}	1.25±0.03^{ab}	1.22±0.05^{ab}	0.025
SGR (% dia^{-1})	2.67±0.13^{b}	3.09±0.02^{a}	3.06±0.04^{a}	3.01±0.08^{ab}	0.018
Protein retention (%)	38.51±1.39^{b}	46.52±1.71^{a}	41.25±0.95^{ab}	44.31±1.98^{ab}	0.029
Energy retention (%)	24.33±0.83^{b}	29.93±1.10^{a}	28.08±0.63^{ab}	28.74±1.26^{ab}	0.019
Feed cost (US$ kg)	1.11±0.06^{a}	0.81±0.05^{b}	0.92±0.04^{b}	0.92±0.07^{b}	0.000

[1]Acronyms according to Table 1.
[2]One-way ANOVA P-values and different superscript letters in the same line indicate a significant difference ($P < 0.05$) between treatments in Tukey's test.

Table 3. Whole-body composition and somatic indices (mean ± standard error) of *Colossoma macropomum* fed experimental organic diets.

Parameters[1]	Diets[2]				P-value[3]
	CL	BN	CLBN	FFBN	
Whole-body composition					
Moisture (g kg^{-1})	713.03±7.40^{ab}	692.09±4.63^{b}	719.55±4.40^{a}	709.28±4.35^{ab}	0.011
Protein (g kg^{-1})	160.03±4.56	167.86±3.87	153.26±2.68	160.91±2.63	0.062
Lipids (%)	84.34±1.98	88.16±1.67	82.73±2.11	86.13±1.59	0.222
Ash (%)	39.83±1.34	44.17±2.80	42.51±1.96	39.60±0.58	0.272
Somatic indices					
Viscerosomatic index (%)	8.49±0.52	8.99±0.31	8.87±0.24	9.24±0.27	0.513
Hepatosomatic index (%)	1.70±0.12	1.74±0.07	1.77±0.09	1.78±0.08	0.944
Visceral fat index (%)	2.08±0.24^{c}	2.93±0.23^{ab}	2.18±0.18^{bc}	3.22±0.18^{a}	0.001
Intestine length/Total length	1.61±0.07	1.51±0.08	1.68±0.03	1.66±0.03	0.213

[1]Values expressed in wet basis.
[2]Acronyms according to Table 1.
[3]One-way ANOVA P-values and different superscript letters on the same line indicate a significant difference (P < 0.05) between treatments via the Tukey's test.

Table 4. Blood parameters (mean ± standard error) of juvenile Colossoma macropomum fed the experimental organic diets. RBC: Red blood cell counts, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LRA: Leukocyte respiratory activity, WBC: White blood cell count.

Parameters	Diets[1]				P-value[2]
	CL	BN	CLBN	FFBN	
Hematologic parameters					
Hematocrit (%)	27.67±1.68	27.22±1.41	26.06±1.10	29.81±1.92	0.401
Hemoglobin (g dL^{-1})	5.24±0.30	6.00±0.37	5.82±0.24	5.95±0.30	0.286
RBC (10^{6} µL^{-1})	1.35±0.07	1.37±0.10	1.27±0.06	1.55±0.09	0.118
MCV (fL)	205.68±9.67	203.91±11.94	206.99±10.06	193.35±7.90	0.763
MCHC (g dL^{-1})	19.51±1.55	22.53±1.79	23.92±1.86	20.41±1.15	0.221
MCH (pg)	39.54±2.82	44.55±3.97	42.38±2.03	38.89±1.64	0.437
Biochemical parameters					
Glucose (mg dL^{-1})	63.91±2.79	70.91±4.17	63.14±5.36	73.77±4.77	0.253
Cortisol (ng ml^{-1})	34.72±7.63^{ab}	22.89±.02^{b}	31.92±4.49^{ab}	51.86±6.00^{a}	0.025
Triglycerides (mg dL^{-1})	259.12±22.27^{b}	266.82±26.11^{b}	266.26±12.22^{b}	412.49±35.89^{a}	0.000
Cholesterol (mg dL^{-1})	119.21±7.22	131.96±8.79	126.07±9.79	132.50±8.25	0.665
Total protein (g dL^{-1})	2.61±0.18	2.98±0.32	2.99±0.14	2.97±0.15	0.504
ALT (U L^{-1})	7.64±3.06	3.88±1.53	8.73±3.56	3.88±2.11	0.449
AST (U L^{-1})	64.85±4.99	104.80±20.50	114.70±24.90	93.10±16.20	0.287
Innate immunity parameters					
LRA (mg mL^{-1})[9]	2.27±0.09	2.40±0.06	2.37±0.06	2.42±0.08	0,483
Thrombocytes (µL x 10^{3})	1.36±0.34	1.31±0.33	0.68±0.34	1.98±0.42	0,121
WBC (µL x 10^{3})[10]	28.68±1.92^{a}	27.88±2.31^{ab}	19.05±1.64^{b}	25.55±3.31^{ab}	0,013
Lymphocytes (µL x 10^{3})	18.49±2.24^{ab}	18.22±1.80^{ab}	10.58±1.35^{b}	21.37±3.75^{a}	0,017
Monocytes (µL x 10^{3})	10.19±1.82	9.20±1.19	8.36±0.88	7.93±1.42	0,668
Neutrophils (µL x 10^{3})	0.00±0.00	0.46±0.16	0.11±0.11	0.20±0.14	0,063

[1]Acronyms according to Table 1.
[2]One-way ANOVA P-values and different superscript letters on the same line indicate a significant difference (P < 0.05) between treatments using the Tukey's test.

Table 5. Digestive enzyme activity (mean ± standard error) of juvenile Colossoma macropomum fed experimental organic diets.

Parameters	Diets[1]				P-value[2]
	CL	BN	CLBN	FFBN	
Acid proteases (U L^{-1})	6,538.44 ± 1,185.16	5,809.12 ± 1,680.22	5,228.65 ± 1,267.16	8,395.89 ± 1,254.94	0.392
Trypsin (U L^{-1})	271.34 ± 36.09^{b}	309.26 ± 50.63^{ab}	389.30 ± 29.88^{ab}	450.19 ± 44.73^{a}	0.023
Lipase (U L^{-1})	62.33 ± 3.47	53.57 ± 5.35	47.12 ± 3.67	50.37 ± 7.61	0.189
Amylase (U L^{-1})	86,483.03 ± 12,740.83	72,884.85 ± 7,898.43	120,589.80 ± 12,466.04	120,889.08 ± 16,198.24	0.040

[1]Acronyms according to Table 1.
[2]One-way ANOVA P-values and different superscript letters on the same line indicate a significant difference (P < 0.05) between treatments using the Tukey's test.

the same level of digestible protein, had a protein retention of 37.64% (Buzollo *et al*. 2019), a lower value than what was found for all the diets evaluated in the present study. The high retention of nutrients in experimental diets, compared to the results in the literature, are indicators of the high biological value and nutritional quality of the ingredients used in the formulation of organic diets.

Fish fed the BN diet showed greater growth, energy, and protein utilization in comparation to the CL diet. BN diet has not caused changes in physiological homeostasis, metabolism, and body composition. The defatted Brazil nut meal, the main ingredient of this diet, has high concentrations of amino acids, unsaturated fatty acids, bioactive compounds with antioxidant properties, micronutrients, and a low concentration of substances associated with antinutritional factors (Santos *et al*. 2013). On the other hand, fish on the CL diet had worse growth performance and energy and protein utilization. This can be related to the presence of antinutritional factors in the cassava leaf, such as tannins, phytates, cyanogenic acids, lectins, and trypsin inhibitors (Olude *et al*. 2021).

The high concentration of cassava leaf meal in the CL diet reduced trypsin activity, directly affecting the use of nutrients and the growth performance of the animals. In addition to the loss of growth, changes in the color of the fish were also observed, a pattern observed in other studies with the species fed with cassava leaf meal (Matos-Dantas *et al*. 2024) (Figure 2). The results observed in this study were similar to those reported for post-larvae *Labeo rohita* when fed with 390 g kg^{-1} of cassava leaf meal a reduction in weight gain and metabolic activity, and an increase in cellular and oxidative stress (Olude *et al*. 2021). Antinutritional factors can negatively influence intake and absorption of nutrients and energy from diets by altering taste, agglutination, formation of stable complexes and/or inhibition of digestive enzyme activity (Dongmeza *et al*. 2009; Omnes *et al*. 2017).

The concentration of trypsin inhibitors in the cassava leaf increases with the age of the plant and varies according to the variety, with values between 0.57 and 3.28 IU mg^{-1} (Wobeto *et al*. 2006). Although heat reduces the activity of trypsin inhibitors (Agrahar-Murugkar and Jha 2010), the thermal processing during the extrusion of the feed was not sufficient to nullify their action in the CL in the present study. Another antinutritional factor present in ground cassava leaf is hydrogen cyanide (HCN), which, in high concentrations, induces asphyxia and prevents tissue utilization of oxygen by inhibiting the cellular respiratory enzyme, cytochrome oxidase, and causing death of the animals (Shwetha and Hosetti 2009). Although most of the HCN is removed during the grinding and drying, residual amounts of this compound can have a chronic effect. During cyanide detoxification, the use of methionine as a sulfur donor in the reaction with thiosulfate to form thiocyanate can result in reduction of this essential amino acid (Oke 1978; Olude *et al*. 2021). Recent studies have indicated that optimal methionine levels are related to increased immune response in fish cells (Azeredo *et al*. 2017). Cassava leaves are deficient in sulfur amino acids (Ravidran 1993). The sum of these factors together with the observed enzymatic inhibition may have contributed to the alterations observed in the leukocyte count and number of lymphocytes in the fish fed the CL and CLBN diets, and, in the latter, these alterations were not accompanied by an decrease in growth performance.

Amino acids play essential roles in different tissues of animals, from muscle synthesis to acting in defense cells. Unbalanced diets can cause reduced growth, changes in fat deposition, immunosuppression, and changes in the formation of muscle cells in fish (Pereira *et al*. 2017; Buzollo *et al*. 2019). The lysine concentrations in the CL, BN and CLBN diets marginally met the requirement of the species (Table S1), while the FFBN diet exceeded the recommended value by 3 g kg^{-1} (NRC 2011; Marchão *et al*. 2020). This excess, added to the higher concentration of saturated fat in ingredients of animal origin (NRC, 2011), may have contributed to the greater deposition of cavity fat and triglycerides in fish fed the FFBN diet.

Excess protein and peptides in diets can induce the release of cortisol (Arnold-Reed and Balment 1994), which in turn stimulates the body to respond to the effects of the stressors, thereby influencing and regulating the use of nutrients from the diet or from tissues through gluconeogenesis and lipolysis pathways (Stachowicz and Lebiedzinska 2016). Despite the

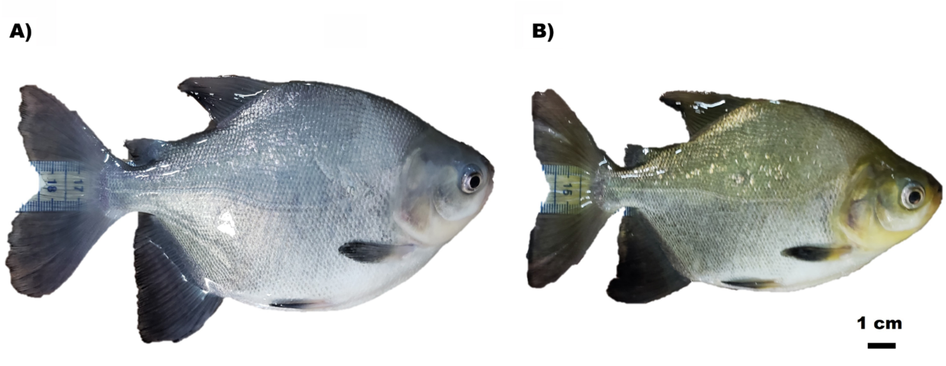

Figure 2. Juvenile tambaqui fed organic diets for 60 days. **A)** Fish fed with a diet based on Brazil nut; **B)** Fish fed a diet based on cassava leaf meal.

increase in levels of this corticosteroid hormone in fish fed the FFBN diet, the observed values (< 55 ηg ml^{-1}) for all diets were close to those described for the species, without the influence of stressors (Ruiz-Jarabo *et al*. 2020; Queiroz *et al*. 2022). In addition to changes in metabolic compound levels, excess protein can negatively influence tambaqui production costs (Dos Santos *et al*. 2023).

The feed costs associated with the organic experimental diets were comparable to the current average market prices of commercial extruded feeds containing 32% crude protein for omnivorous fish, thereby positioning these formulations within a competitive cost range. According to FAO (2024), the average global export value of aquatic animal products reached USD 2.7 per kilogram (live weight equivalent) in 2022. The cost of the least efficient diet in this study (CL) per kg of fish produced represented 40.74% of this value. From an economic perspective, despite the CL diet incurring the highest production cost among the treatments, organic diets formulated with Amazonian by-products present like a promising alternative. These diets hold considerable potential to enhance the economic viability of small-scale aquaculture in the Amazon region, particularly when integrated into circular bioeconomy frameworks that promote local resource use and value chain sustainability.

In addition to growth and economic parameters, sensory attributes such as flavor, texture, color, and odor of fish fillets are decisive factors in consumer purchasing behavior and should be considered in sustainable feed development strategies (Freitas *et al*. 2020). Use of organic ingredients in aquafeeds can enhance the sensory quality of fish, leading to a milder taste profile and more visually appealing fillets (Mauracher *et al*. 2013; Calanche *et al*. 2020). Therefore, further studies are essential to systematically assess the fillet quality of fish raised on organic diets, particularly focusing on how different by-product combinations influence organoleptic parameters and consumer preferences.

CONCLUSION

The results of this study indicate that the use of by-products from Amazonian value chains in the production of feed, in accordance with organic certification standards, is viable from the perspective of both productive performance and animal welfare. The use of these diets in organic production systems has the potential to improve the sustainability of family aquaculture and contribute to the circular bioeconomy in the Amazon.

ACKNOWLEDGMENTS

The authors would like to thank the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - CAPES / *Fundação de Amparo à Pesquisa do Estado Amazonas* - FAPEAM for the research grants conceded to Paulo A. de Medeiros (88887.641288/2021-00) and the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* - CNPQ for the research grant (312492/2021-9) conceded to Ligia U. Gonçalves. This research was also financially supported by FAPEAM PAINTER+ / 116/ 2023), and INCT ADAPTA II / CNPq (465540/ 2014-7) / FAPEAM (062.1187 / 2017).

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RECEIVED: 16/12/2024
ACCEPTED: 29/04/2025
ASSOCIATE EDITOR: Marcos Tavares-Dias
DATA AVAILABILITY: The data supporting the findings of this study are available upon reasonable request from the corresponding author, Paulo Adelino de Medeiros.

SUPPLEMENTARY MATERIAL

Medeiros *et al*. Aquaculture feeds produced with Amazon by-products in accordance with organic certification standards: effect on growth and health of *Colossoma macropomum*

Table S1. Amino acid composition of experimental diets.

Amino acid composition (g kg^{-1})	Diets[1]			
	CL	BN	CLBN	FFBN
Arginine	17.23	22.93	21.12	20.41
Phenylalanine	13.28	11.90	12.98	12.83
Histidine	5.87	5.91	6.14	6.90
Isoleucine	11.83	10.69	12.21	11.86
Leucine	21.13	19.52	21.18	20.61
Lysine	15.78	13.81	15.71	18.89
Methionine	6.15	10.31	8.31	10.20
Taurine	0.54	0.50	0.62	0.68
Threonine	12.82	11.14	12.58	12.40
Valine	16.34	15.42	16.87	15.05
Alanine	19.67	18.19	19.55	20.40
Aspartic Acid	23.21	19.91	23.87	24.45
Glutamic Acid	35.28	40.75	39.98	39.03
Cystine	7.73	11.65	6.85	4.09
Glycine	24.17	25.58	25.45	24.35
Hydroxyproline	5.49	6.40	5.89	5.05
Proline	16.70	17.20	17.86	16.39
Serine	16.36	15.54	16.32	13.26
Tyrosine	11.17	9.98	10.83	11.74
Sum of amino acids	280.78	287.30	294.31	288.59

[1]CL: diet based on cassava leaf meal. BN: diet based on defatted Brazil nut meal. CLBN: diet based on cassava leaf meal and Brazil nut meal. FFBN: fish residue, black soldier fly larvae and defatted Brazil nut meals.