

Farming tambaqui (*Colossoma macropomum*) using byproducts from the Amazon bioeconomy: nutritional composition, digestibility and attractiveness

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ABSTRACT

The Amazon bioeconomy produces byproducts that have potential as ingredients in aquaculture feed. This study aims to evaluate nutritional composition, digestibility, and attractiveness of six regional byproducts – Artisanal Fish Waste Meal (FM), Defatted Brazil Nut Meal (BN), Cassava Leaf Meal (CL), Decaffeinated Guarana Bagasse (GB), Defatted Murumuru Meal (MU), and Defatted Ucuuba Meal (UC) – for use as tambaqui (*Colossoma macropomum*) feed. The byproducts had diverse nutrient profiles, with FM providing significantly more digestible protein and energy, followed by BN and CL. Attractiveness tests indicate that CL and BN are approached more rapidly and consumed more readily: BN and CL have potential as plant-based alternatives, due to their digestibility and attractiveness to the fish. In contrast, GB and UC were limited in both digestibility and feeding stimulation. We suggest that the byproducts FM, BN, and CL can easily be integrated into tambaqui diets, supporting sustainable aquaculture practices in the Amazon. Additional research can determine how to optimize processing and inclusion to fully exploit the functional properties of these ingredients while ensuring cost-effective and environmentally friendly fish farming.

KEYWORDS: aquaculture; Brazil nut; cassava; circular bioeconomy; feeds

Criação de tambaqui (*Colossoma macropomum*) com subprodutos da bioeconomia amazônica: composição nutricional, digestibilidade e atratividade

RESUMO

A bioeconomia da Amazônia gera subprodutos agroindustriais com potencial para utilização como ingredientes na alimentação aquícola. Este estudo avaliou a composição nutricional, a digestibilidade e a atratividade de seis subprodutos regionais – Farinha de Resíduos de Peixe Artesanal (FM), Farinha Desengordurada de Castanha do Brasil (BN), Farinha de Folha de Mandioca (CL), Bagaço Descafeinado de Guaraná (GB), Farinha Desengordurada de Murumuru (MU) e Farinha Desengordurada de Ucuuba (UC) – para uso na alimentação do tambaqui (*Colossoma macropomum*). Os subprodutos apresentaram perfis nutricionais variados, com a FM fornecendo níveis significativamente mais altos de proteína e energia digestível, seguida pela BN e CL, que ofereceram aproximadamente 200 g kg⁻¹ de proteína digestível. Os testes de atratividade mostraram variações significativas, com CL e BN promovendo menores tempo de aproximação e maiores taxas de ingestão dos grânulos. BN e CL mostraram-se promissoras como fontes alternativas vegetais, devido à boa digestibilidade e atratividade. Em contrapartida, GB e UC apresentaram limitações relacionadas à digestibilidade e ao estímulo alimentar, possivelmente devido à presença de fatores antinutricionais. Os resultados destacam o potencial de integração de FM, BN e CL em rações para tambaqui, contribuindo para práticas aquícolas sustentáveis na Amazônia. No entanto, são necessárias pesquisas adicionais para otimizar os processos de preparo e os níveis de inclusão, maximizando as propriedades funcionais dos ingredientes e promovendo a piscicultura sustentável.

PALAVRAS-CHAVE: castanha do Brasil; aquicultura; mandioca; bioeconomia circular; ração

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INTRODUCTION

Sustainable development strategies of the Amazon region favor using bioeconomy (Ellwanger *et al.* 2023). Within the bioeconomy sector, fish aquaculture has been gaining importance and reliability, especially considering the adverse effects of climate extremes on traditional fishing (Furtado *et al.* 2023). A critical challenge for traditional Amazonian communities is to develop sustainable production systems that compensate for nutritional deficits caused by declining fish stocks (Tregidgo *et al.* 2021). Transitioning from fishing to aquaculture offers an alternative pathway for economic and social development in regions where fish stocks no longer meet population demands (FAO 2024).

Despite favorable climate, abundant water, and a diversity of species, aquaculture in the Amazon faces significant challenges. Production costs, particularly those associated with feed, hinder the competitiveness of the sector (Lima *et al.* 2020). Regional research efforts have evaluated alternative feed ingredients to increase productivity, reduce costs, and diversify raw materials for fish feed processing (Felix and Silva *et al.* 2020; Hilsdorf *et al.* 2021; Monteiro dos Santos *et al.* 2022). The use of byproducts as feed ingredients depends on several factors, including production volume, cost, nutritional composition, palatability, and digestibility for the target species (Glencross 2020).

Tambaqui *Colossoma macropomum* Cuvier (1818), a native omnivorous fish, is the most common species cultivated in Amazonian aquaculture (Lima *et al.* 2020). Its adaptability to diverse production systems, tolerance to high stock densities, and ability to withstand low dissolved oxygen levels make it a good candidate for aquaculture expansion (Wood *et al.* 2017; Dos Santos *et al.* 2020; Frisso *et al.* 2020; Izél-Silva *et al.* 2020). Tambaqui are remarkably able to consume a variety of items from regional sources, including agriculture, aquaculture, and fisheries (Guimarães *et al.* 2014; Aride *et al.* 2017; Buzollo *et al.* 2018; Aride *et al.* 2020).

Diets including byproducts from cassava (*Manihot esculenta* Crantz) processing have sustainable benefits with juvenile tambaqui, yielding growth performance comparable to commercial feeds (Aride *et al.* 2016; Matos-Dantas *et al.* 2024). Also, residues from non-timber extraction (e.g., Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.)) are viable potential alternatives (Santos *et al.* 2010; Da Costa *et al.* 2022). Limited information is available regarding the tambaqui ability to digest and assimilate energy, nutrients, and minerals from these ingredients to make them viable and useful alternatives.

Other agro-industrial byproducts from the rich biodiversity of the Amazon have potential as feed because of their nutrient profiles, such as cassava leaf meal, defatted murumuru palm (*Astrocaryum murumuru* Mart.) meal, defatted ucuuba (*Virola surinamensis* Rol.) a nutmeg meal, and decaffeinated guarana (*Paullinia cupana* Kunth) bagasse (Hisano *et al.* 2013; Medeiros *et al.* 2018). However, in addition to nutritional composition, palatability and digestibility *in vivo* – essential characteristics for aquaculture feeds (Glencross 2020) – must be tested. Given

the tendency of the tambaqui to accept a variety of food items, the large-scale availability of agro-industrial byproducts in the region, and the importance of accessible feed solutions for family fish farming, this study measured nutritional composition, and tested digestibility and attractiveness of six regional byproducts as ingredients in the diet of tambaqui (*Colossoma macropomum*).

MATERIALS AND METHODS

Ethics authorizations

Experiments to test digestibility and attractiveness were carried out at the Aquaculture Station of the Instituto Nacional de Pesquisas da Amazônia (INPA) under the authorization of the Ethics Committee Use of Animals in Research of INPA (No. 020/2020, SEI 01280.000410/2020-76).

Experimental design and fish acclimatization

A completely randomized experimental design with seven treatments (a reference diet, and six test diets), in triplicate, was carried out in conical tanks (200 L volume) positioned to avoid the fish having visual contact with people during handling and data collection (Figures 1a-c). Tanks had continuous aeration, and daily renewal of 30% of the water volume.

Once fish were placed in the tanks, acclimatization to the experimental setup lasted ten days, during which time they were provided feed twice a day with commercial feed (32% crude protein) until apparent satiety. During acclimatization, feces from the first seven days of collection were discarded because intestinal transit time of the tambaqui (Silva *et al.* 2003, Reis *et al.* 2009) allowed us to avoid influence of diet prior to the experiment. Two weeks of collection is the period during which the apparent digestibility coefficients of a new diet offered reach stable levels in rainbow trout *Oncorhynchus mykiss* Walbaum (1792) (Staessen *et al.*, 2020). We defined a 30-day fecal collection period based on intestinal transit time, digestion stabilization, and sample volume required for analysis.

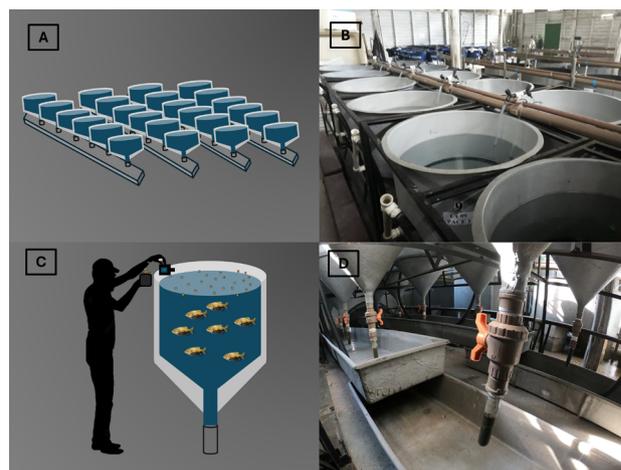


Figure 1. Experimental design. **A)** Graphical representation of the structure of conical tanks; **B)** Digestibility tank system; **C)** Graphical representation of the attractiveness data collection; **D)** Fish feces collection system.

Origin, processing, and yield of byproducts

Raw materials were sourced from agro-industries in the Brazilian Amazon: artisanal fish waste meal (FM), cassava leaf meal (CL), and decaffeinated guarana bagasse (GB) from Maués, Amazonas, Brazil; defatted Brazil nut meal (BN), defatted murumuru meal (MU), and defatted ucuuba meal (UC) were collected from Ananindeua, Pará, Brazil (Table 1). Ingredients were dried and ground to an average grain size of 1 mm using a hammer mill (MSC280, Vieira, SP, Brazil).

Analysis of nutritional composition

Dry matter was obtained by the gravimetric method of heating the samples to 105 °C (method 934.01) in an oven (model 3632, Alfamare, MG, Brazil). The ethereal part was extracted using petroleum ether solvent extraction (method 920.39) in a goldfish extractor (model TE-044-8/50, Tecnal, SP, Brazil). The ash was then incinerated at 450 °C (942.05).

Nitrogen was measured using the Kjeldahl method (AOAC 1995, method 984.13) in a nitrogen distiller (model TE-0364, Tecnal, SP, Brazil). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were calculated following Mertens *et al.* (2002), both ash-free, in fiber determinator (model TE-149, Tecnal, SP, Brazil). Lignin was calculated using the ADF residue, following the method proposed by Robertson and Von Soest (1981).

Phosphorus concentration was measured by spectrophotometry (method 965.17) and calcium by titration of dry ash made alkaline with ethylenediaminetetraacetic acid (EDTA) (method 927.02). Gross energy was calculated by incinerating the samples in a bomb calorimeter (model C-2000,

Table 1. Processing methods and yield after dehydration and/or extraction of oils from raw materials used for the preparation of fish diets.

Byproduct	Processing	Yield ¹ (%)
Artisanal Fish Waste meal processed at low temperature (FM)	Processing residues and whole fish not fit 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 steel oven. Adapted to Lourenço <i>et al.</i> (2011)	33.6
Cassava leaf meal (CL)	Leaves and the upper third of the stem of the plant were ground and dried in the shade for 72 h, as recommended by Hisano <i>et al.</i> (2013).	20.0
Decaffeinated Guarana bagasse (GB)	Dehydration of the seeds (<12% moisture), followed by crushing (3 – 5 mm) and hydroalcoholic extraction of guaranine, as described by Santana <i>et al.</i> (2019).	35.0
Defatted Brazil nut meal ² (BN)		32.8
Defatted Murumuru meal ² (MU)	Extraction of the lipid fraction of almonds by mechanical pressing at 1.22 x 106 N m ² .	32.0
Defatted Ucuuba meal ² (UC)		38.6

¹ Considering the moisture content of approximately 12% in the byproduct.

² Personal communication from Amazon Oil Company©.

IKA, Guangzhou, China). Amino acids were measured using high-performance liquid chromatography (HPLC) (model 1290 infinity, Agilent Technologies, CA, USA).

Chromium oxide concentration was determined using the method proposed by Furukawa and Tsukahara (1966). All byproducts were measured following the Association of Official Analytical Chemists (AOAC 2005).

Determination of Apparent Digestibility

Digestibility (as the apparent digestibility coefficient, ADC) was measured from the feces with an *in vivo* experiment using chromium oxide labeled diets in the feed (Bureau and Hua, 2006). Test diets were formulated with 30% of the test ingredients in the reference diet (Table 2), processed in an extruder machine with a 4 mm matrix (model MX-80, Inbramaq, SP, Brazil) at an average temperature of 100°C, and dried in an oven at 60°C for eight hours (model TE-394/3, Tecnal, SP, Brazil).

In total, 240 tambaqui juveniles (58.62 ± 0.69 g) in 21 tanks, and individual feces collection system. Fish were fed twice daily (08:00 and 18:00 h) until apparent satiety. Tanks were cleaned and siphoned, and 15% of the water was renewed, 1 h after each feeding to avoid contamination of the

Table 2. Formulation and nutritional composition in dry matter (g kg⁻¹) of the reference diet for the digestibility and attractiveness experiments.

Ingredients	Reference diet (g kg ⁻¹)
Commercial fish meal ¹	50.0
Meat and bone meal ¹	42.5
Poultry byproduct meal ¹	110.0
Corn gluten ¹	100.0
Soybean meal ¹	360.0
Corn meal ¹	320.0
Soybean oil ²	5.0
DL-Methionine ¹	2.5
Vitamin and mineral supplement ³	5.0
Chromium Oxide	5.0
<i>Nutritional composition</i>	
Dry matter	908.3
Crude protein	402.1
Ethereal extract	137.4
ADF ⁴	52.3
NDF ⁵	112.8
Lignin	10.6
Ash	134.4
Calcium	31.8
Phosphorus	14.2
Gross energy (MJ kg ⁻¹)	16.5

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³ Enrichment per kg: Vitamin A 5600 IU; Vitamin D 1400 IU; Vitamin E 40 IU; Vitamin K3 1.4 mg; Vitamin B1 4 mg; Vitamin B2 8 mg; Vitamin B6 4.8mg; Vitamin B12 10.5 mg; Folic Acid 0.72 mg; Choline 172 mg; Biotin 0.4 mg; Niacin 13.6 mg; Vitamin C 300 mg; Lysine 950 mg; Methionine 1250 mg; Zinc 48 mg; Cu 5 mg; Fe 20 mg; Mg 20 mg; I 0.28 mg; If 0.2 mg; Antioxidant BHA 2.42 mg.

⁴ Fiber in ash-free acid detergent.

⁵ Ash-free neutral detergent fiber.

collected material with leftover food. At that time, feces were removed while siphoning to avoid sample contamination by uneaten feed. Feces were collected (for ADC measurement) prior to feeding each day (07 :00 and 17:00 h), and was immediately stored at -20°C. The collection period lasted 30 days. Feces samples were lyophilized (model LC3000, Terroni, SP, Brazil) to determine nutritional composition.

Water physical and chemical characteristics were within the comfort levels for tambaqui (Wood *et al.* 2017; Barroso *et al.* 2020; Carmo-Neves *et al.* 2020): temperature (27.71 ± 1.18 °C), dissolved oxygen (4.33 ± 1.14 mg L⁻¹), conductivity (132.08 ± 38.00 µS cm⁻¹), pH (6.93 ± 0.35), total ammonia (1.74 ± 1.05 mg L⁻¹), nitrite (0.23 ± 0.31 mg L⁻¹), alkalinity (47.98 ± 7.60 mg.L⁻¹ of CaCO₃) and hardness (11.15 ± 6.83 mg.L⁻¹ of CaCO₃). Significant concentrations of ionized ammonia (NH₃) were not detected.

ADC was calculated following Bureau and Hua (2006). For the ADC values of the six ingredients, the following formula was used: ADC of the ingredients (%) = ADC_{DIET TESTE} + [(ADC_{DIET TESTE} - ADC_{DIET REFERENCE}) × (0.7 × D_{REFERENCE} / 0.3 × D_{INGREDIENT}), where D_{REFERENCE} and D_{INGREDIENT} are nutrients (g kg⁻¹) or gross energy (MJ kg⁻¹) in the reference diet and test ingredient, respectively.

Attractiveness analysis

Behavior to determine attractiveness was filmed beginning three days after initiation of test diets (Glencross 2020). Fish were acclimated to the camera at feeding time for 10 days prior to data collection. For 15 days, 30 feedings were filmed, and attractiveness was calculated for each diet, following Kasumian and Sidorov (2012) and Hattori *et al.* (2021), but with 30 s interval of filming. High resolution filming (camera model Hero 5 Black, Go Pro, CA, USA) began 30 s after 3 g of feed (70 granules) was placed in the tank. Videos were analyzed in individual frames every 0.03 seconds using video editing Animotica[®] software. After analyzing the images, behavior was measured as follows: approximation time (AT: seconds until capture of the first grain), capture reactions (CR: number of projections or mouth openings with or without consumption during 30 s), grain intake (GI: percentage of grains that were ingested in 30 seconds in relation to the total offered, and grain ejection (GE, number of grains consumed and ejected back into the water).

Statistical analysis

Digestibility and attractiveness were analyzed among the feeding treatments using analysis of variance, assuming that the residuals meet the test assumptions for parametric analysis. When not, we used their non-parametric counterpart (Kruskal-Wallis). To rank treatments we used the parametric Tukey test or its non-parametric version. All analyses were performed with a significance level of 5% using Statistica 7[®] software.

RESULTS

There was wide variation in the proximal composition among the byproducts used to prepare the tested diets (Table 3). The crude protein content of the evaluated diets ranged from 108 to 556 g kg⁻¹, and the ether extract content was greater than 100 g kg⁻¹ in FM, BN, MU, and UC. Ingredients of plant origin had NDF concentrations greater than 300 g kg⁻¹.

Digestible protein (DP) of FM was somewhat more than twice that of BN and CL, all of which were much greater than GB, MU and UC (Table 4). Digestible ethereal extract (fats)

Table 3. Nutritional composition of dry matter compared among byproducts from the Amazon region and used in this study.

	Amazon bioeconomy byproducts ¹					
	FM	BN	CL	GB	MU	UC
Nutritional composition (g kg⁻¹)						
Dry matter	972.2	907.0	852.4	689.4	918.2	974.1
Crude protein	556.2	227.6	261.0	129.8	103.1	108.7
Ethereal extract	103.6	181.9	42.9	42.3	170.9	114.8
ADF ²		255.1	283.0	348.4	342.3	353.7
NDF ³		382.6	404.1	506.4	601.0	540.4
Lignin		68.1	102.7	70.3	176.9	65.6
Ash	193.3	23.3	51.6	26.1	21.1	69.8
Calcium	65.7	1.9	3.6	2.8	1.6	3.2
Phosphorus	27.8	4.4	2.8	1.7	3.2	1.8
Crude energy (MJ Kg ⁻¹)	20.5	21.9	20.1	20.2	22.2	21.1
Amino Acids composition (g kg⁻¹)						
Arginine	36.4	28.1	13.5	8.9	5.3	9.6
Histidine	12.5	5.0	4.6	3.2	1.0	2.0
Isoleucine	22.3	7.4	9.7	5.8	2.6	4.9
Leucine	39.3	16.0	19.1	9.1	4.6	7.4
Lysine	47.9	6.6	12.5	6.7	1.4	4.0
Methionine	18.6	14.5	4.4	2.3	2.1	2.2
Phenylalanine	22.9	9.3	12.0	6.5	3.2	5.6
Taurine	1.7	0.6	0.2	0.0	0.0	0.2
Threonine	26.1	6.8	10.9	5.6	2.5	4.0
Valine	28.6	11.1	12.6	6.5	3.6	6.5
Alanine	40.5	8.9	14.3	6.1	3.3	5.3
Aspartic Acid	42.5	18.5	21.1	13.9	6.0	10.5
Cystine	28.6	13.8	2.9	2.0	0.9	1.0
Glutamic Acid	75.8	43.3	26.6	21.7	14.3	17.1
Glycine	50.3	10.4	12.2	7.1	3.2	5.3
Hydroxyproline	13.9	0.4	1.5	1.2	0.3	1.5
Proline	24.6	7.7	9.0	5.6	2.5	5.3
Serine	25.4	10.0	11.2	6.6	2.9	5.7
Tyrosine	18.2	6.3	9.1	4.5	2.1	3.5
Total amino acids	575.9	224.5	207.4	123.1	61.8	101.7

¹Byproducts: Fish waste meal processed at low temperature (FM); Defatted Amazon nut meal (AN); cassava leaf meal (CL); Decaffeinated guarana bagasse (GB); Defatted murumuru meal (MU); Defatted ucuuba meal (UC).

²Fiber in acid detergent.

³Fiber in neutral detergent.

Table 4. Apparent digestibility coefficient, digestible nutrient levels (g kg⁻¹), and digestible energy (MJ kg⁻¹) of test ingredients for *Colossoma macropomum*. Test ingredients: fish waste meal processed at low temperature (FM); defatted Amazon nut meal (BN); cassava leaf meal (CL); decaffeinated guarana bagasse (GB); defatted murumuru meal (MU); defatted ucuuba meal (UC). Variables: Apparent digestibility coefficient = Dry matter (ADC DM); Crude protein (ADC CP); Ethereal extract (ADC EE); Phosphorus (ADC PP); Gross energy (ADC GE); Digestible protein (DP); Digestible ether extract (DEE); Digestible phosphorus (DPP); Digestible energy (DE).

Variables	ADC and digestible levels of test ingredients						P
	FM	BN	CL	GB	MU	UC	
Apparent digestibility coefficient (ADC)							
DM	0.79 ^a	0.44 ^{bc}	0.37 ^c	0.65 ^{ab}	0.32 ^c	0.35 ^c	0.000
CP	0.93	0.85	0.79	0.93	0.72	0.84	0.348
EE	0.86 ^a	0.84 ^a	0.31 ^b	0.85 ^a	0.86 ^a	0.81 ^a	0.000
PP	0.99	0.91	0.71	0.74	0.56	0.30	0.082
GE	0.86 ^a	0.68 ^{bc}	0.62 ^c	0.80 ^{ab}	0.62 ^c	0.60 ^c	0.000
Nutrients and digestible (D) energy							
DP	517.49 ^a	194.51 ^b	206.70 ^b	120.23 ^c	74.58 ^c	91.45 ^c	0.000
DEE	89.10 ^b	152.72 ^a	13.11 ^d	35.84 ^c	146.97 ^a	93.35 ^b	0.000
DPP	27.41 ^a	4.01 ^b	1.99 ^c	1.30 ^c	1.77 ^c	0.53 ^c	0.000
DE (MJ kg ⁻¹)	17.57 ^a	15.00 ^{abc}	12.46 ^c	16.13 ^{ab}	13.65 ^{bc}	12.63 ^c	0.001

Data expressed as mean ± standard deviation. One-way ANOVA P values and different superscript letters in the same line indicate a significant difference between ingredients in Tukey's test (5% significance).

were lower in the CL than in other treatments (Figure 2c). The digestible phosphorus was 6.8 times higher in FM (27.41 g kg⁻¹) than in BN (4.01 g kg⁻¹), the other ingredients had values below 2 g kg⁻¹ of digestible phosphorus (Figure 2d). The highest digestible energy (DE) values in FM, GB, and BN were 17.57, 16.13, and 15 MJ kg⁻¹, respectively, while the lowest values were 12.46 and 12.63 MJ kg⁻¹ for CL and UC (Figure 2f).

Attractiveness differed among most byproducts (Table 5). No granule ejection was observed. Approach time was much more rapid for CL (1.72 s) than GB (12.90 s). Reactions (CR) were greatest with BN, CL, FM and MU, while ingestion rate (GI) was greatest with BN and CL (Figure 3).

Table 5. Attractiveness indicators of feeds with agro-industrial byproducts from the Amazon for *Colossoma macropomum*. Diets (meal): reference diet (RD); fish waste (FM); defatted Brazil nut (BN); cassava leaf (CL); decaffeinated guarana bagasse (GB); defatted murumuru (MU); defatted ucuuba (UC). Variables: Approximation time (AT); Capture reactions (CR); Grain intake rate (GI).

Variables	Feed attractiveness							P
	RD	FM	BN	CL	GB	MU	UC	
AT (s)	4.4 ^{ab}	1.6 ^{bc}	3.0 ^{bc}	1.4 ^c	12.9 ^a	2.8 ^{ab}	3.1 ^b	0.000
CR (30 s)	30.5 ^b	47.0 ^{ab}	51.0 ^a	42.0 ^{ab}	1.0 ^c	35.0 ^b	38.5 ^{ab}	0.000
GI (% 30 s)	42.2 ^{cd}	54.2 ^b	80.3 ^a	61.5 ^{ab}	1.0 ^d	50.7 ^{bc}	46.1 ^{cd}	0.000

Data expressed as median values (n=3). P values from the Kruskal Wallis Test and different superscript letters in the same row indicate a significant difference in the multiple comparisons test (5% probability).

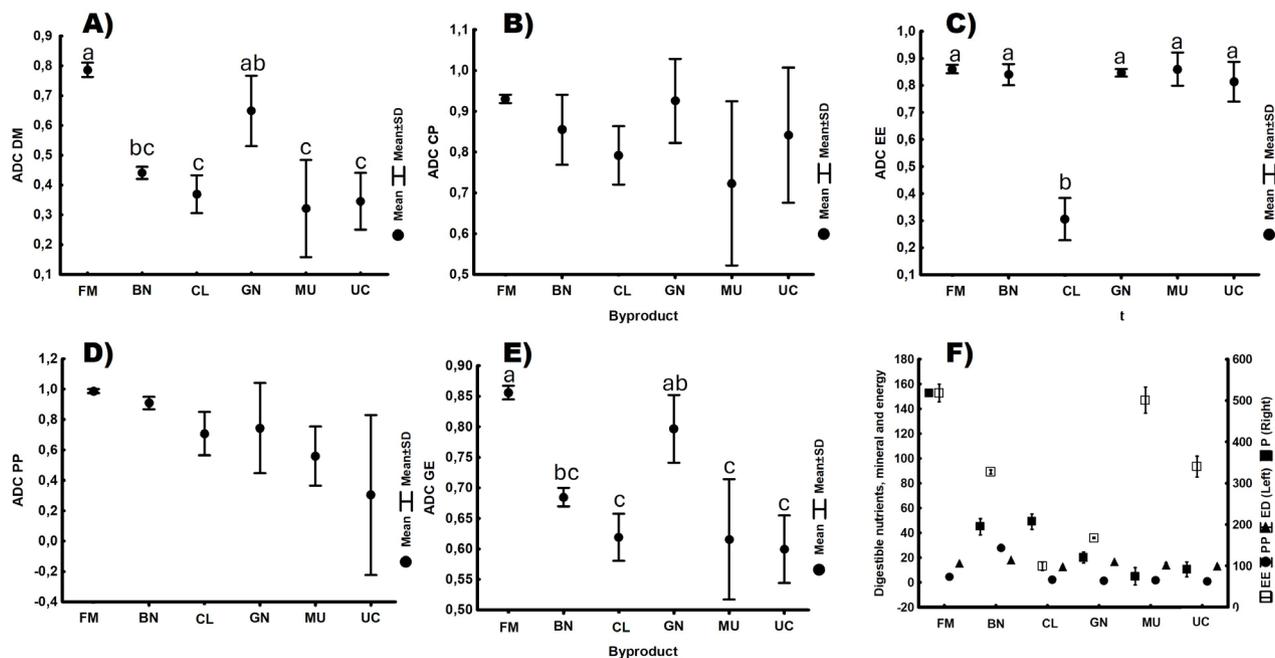


Figure 2. Apparent digestibility coefficient (ADC) of nutrients, minerals, and energy from byproducts of the Amazon bioeconomy. **A)** Dry matter; **B)** Crude protein; **C)** Ether extract; **D)** Phosphorus; **E)** Gross energy; and **F)** Nutrients, minerals, and digestible energy of byproducts.

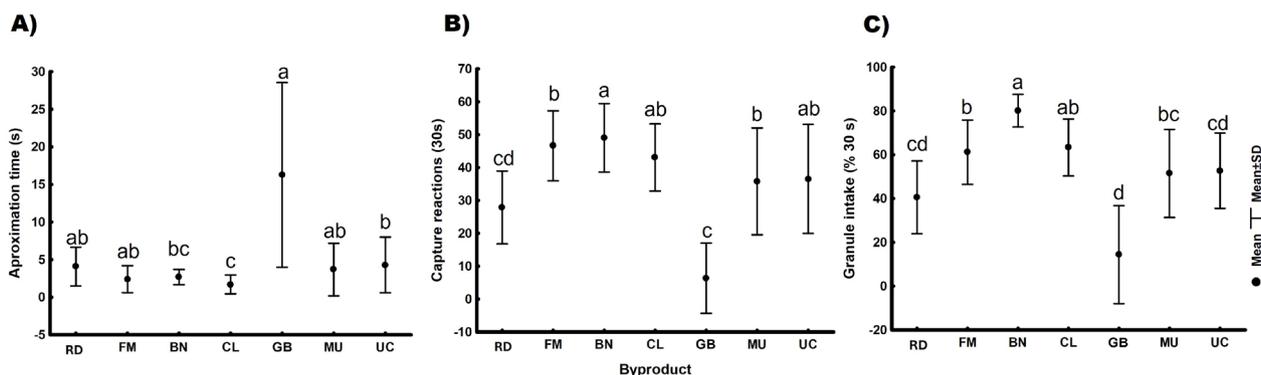


Figure 3. Indicators of attractiveness of byproducts from the Amazon bioeconomy for tambaqui, *Colossoma macropomum*. **A)** Aproximation time; **B)** Capture reactions; and **C)** Granule ingestion rate in 30 seconds

DISCUSSION

Using agroindustrial byproducts in aquaculture shows great potential because fish will readily eat and thrive on a variety of these products, some better than others. Utilization of byproducts (especially those higher in amino acids, e.g., FM, BN, and CL) shows great promise as an alternative for subsidizing small-scale fish production in riverside regions of the Amazon. In addition to supplementing raw materials that may be in short supply, and reducing production costs (Monteiro do Santos *et al.* 2023), this use can favor the diversification of the diet of animals in captivity, meeting a growing demand for healthier diets in inclusive production systems with low environmental impact (Medina *et al.* 2022). Thus, aquaculture in riverside regions can be an important tool in addressing the nutritional and economic deficit of the population, especially in times and regions with reduced fishing productivity (Tregidgo *et al.* 2021).

The inclusion of new ingredients in aquaculture feeds is subject to meeting chemical, technical and market criteria, with nutritional composition being one of the main factors in choosing a raw material (Glencross *et al.* 2020). The low ADC of dry matter in BN, CL, MU, and UC was attributed to the high concentration of non-starch polysaccharides. Most of these compounds are not affected by the heat treatments used in the dehydration of ingredients and the extrusion of feeds. However, advanced processing methods, such as fractionation, aqueous extraction, and the use of exogenous enzymes, may reduce these indigestible components and increase their nutritional value (Drew *et al.*, 2007). In this study, minimal processing was applied to preserve the original composition of the raw materials, highlighting their nutritional potential.

Nutrient supply to farmed fish is influenced by the processing method of the feed ingredients and pellets (Sorensen 2012). The temperature of 70°C used in FM drying may have increased the protein ADC value for tambaqui. Our results are greater than the ADC of 0.78 (Buzollo *et al.*

2018) previously reported for commercial fish waste meal. The processing of waste from artisanal fishing chains and fish processing generates products of high biological value for aquatic animals (Silva *et al.* 2014; Yamamoto *et al.* 2021). Fish meals produced at low drying temperatures (70–90°C) normally have ADC CP above 90% (Hardy and Tacon 2003). Prolonged exposure to high temperatures (> 100°C) causes intense oxidation of amino acids, reducing the digestibility and the chemical and sensory aspects of protein ingredients of animal origin (Zhao *et al.* 2019; Bhat *et al.* 2021). ADC results demonstrate that fishmeal produced in the artisanal process is readily absorbed by fish.

Fish waste is commonly used as an ingredient in aquaculture feed, but the sustainability of these co-products is often debated (Marchi *et al.* 2023; Frohn *et al.* 2024; Sarker *et al.* 2025). Replacing fishmeal with a plant-based ingredient has proven to be viable for tambaqui (Martins *et al.* 2020). Here, the plant-based BN and CL proved to have higher potential for inclusion in tambaqui diets, mainly because of their attractiveness and DP content (and DE in the case of BN). They may replace wheat bran with a high supply of protein and digestible energy (Buzollo *et al.* 2018). There are no other studies on CDA BN in fish, however, whole Brazil nut meal can be included at concentrations of 300 to 350 g kg⁻¹ and maintain tambaqui health and growth rates (Santos *et al.* 2010; Da Costa *et al.* 2022). For Nile tilapia (*Oreochromis niloticus* Linnaeus 1758) shade-dried CL had an ADC of 0.89 (Hisano *et al.* 2013), which was higher than that for protein (0.79) in this study. Thus, these ingredients are potential, alternative, sources of amino acids for the nutrition of tambaqui juveniles.

Amino acids are important stimuli for initiating feeding in aquatic animals, and L-type cysteine, alanine, lysine, and proline induce food search and capture behaviors (Hara, 2006; Yu *et al.*, 2021). L-lysine is an essential amino acid for fish, and because it is highly soluble in water, is among the first to stimulate fish sensory organs (NRC 2011). The higher concentration in CL, in relation to the other byproducts

of vegetable origin, may have contributed to the greater attractiveness of the feed, which was indicated by less time required by the fish to approach this food item.

In addition to amino acids, lipids also modulate food choice and intake in fish (Cardoso *et al.* 2021; Roy *et al.* 2020, 2022). Fat is most recognized for stimulating (texture and smell) eating through the senses, and dietary fats activate the gustatory system (Gaillard and Kinnamon 2019). BN fat has 26% saturated fatty acids, 32% monounsaturated and 42% polyunsaturated fats (Serra *et al.* 2019). The concentration of digestible lipids in BN (~150 g kg⁻¹) increased attraction of fish to the feed, providing more capture reactions and granule ingestion at the beginning of feeding than in animals fed the reference diet based on conventional ingredients.

Corn flour is one of the main sources of dietary energy for fish (Alkoei *et al.* 2024). The molecular structure of corn starch contributes to the extrusion process and the stability of the feed granules (Kannadhasan *et al.* 2011). GB, together with FM and BN, provided DE levels (> 15 MJ kg⁻¹) greater than by corn flour (Buzollo *et al.* 2018). The GB has a low concentration of fats, with the starchy reserves in the seeds responsible for supplying most of the DE (Dalonso and Petkowicz 2021). Inclusion of 300 g kg⁻¹ of GB decreased attractiveness of the feed as this ingredient contains residual alkaloids (Santana *et al.* 2019) and approximately 14% of the tannin (Yamaguti-Sasaki *et al.* 2007; Marques *et al.* 2019). Incorporation of tannin levels above 30 g kg⁻¹ can decrease the cumulative feed intake rates by fish (Omnes *et al.* 2017). The polyphenolic compound gives the feed an unpleasant taste thereby reducing intake (Becker and Makkar 1999), as we found with the longer approach time, low capture rate and ingestion rates by the fish during the first few seconds of feeding.

Antinutritional factors can negatively influence consumption, absorption of nutrients and energy from diets because of flavor, agglutination, formation of stable complexes or inhibition of digestive enzyme activity (Omnes *et al.* 2017). Similar to GB, UC was unattractive to tambaqui. This characteristic, in addition to the low ADC of DM, P, and DP content, demonstrates the low efficiency of both raw materials from the point of view of productive and environmental efficiency as a macro-ingredient. However, it is important to evaluate these byproducts with lower levels of inclusion, as well as in other functional products such as immunomodulatory and nutraceutical additives (Cardoso *et al.* 2021; Copatti *et al.* 2022), given the presence of molecules with antimicrobial, antioxidant, and protective effects against tissue damage (Fukumasu *et al.* 2006; Hiruma-Lima *et al.* 2009; Cordeiro *et al.* 2019).

CONCLUSIONS

We conclude that FM, BN and CL have the greatest potential for application in the manufacture of diets for family fish farming of tambaqui *C. macropomum* by providing easily

absorbed proteins, and they have chemical characteristics that are more attractive and stimulate feed ingestion. Further studies evaluating processing techniques and inclusion levels are needed to make the most of the functional potential of the ingredients and promote healthy growth of the fish during farming.

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