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Practical Diets Formulated With Different Plant-Based Energy Ingredients Modulate Nutrient Utilization and Gut Morphology in Tambaqui (*Colossoma macropomum*)

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ABSTRACT

Despite the increasing use of plant-based energy ingredients in aquaculture, few studies have evaluated the digestibility of practical diets formulated with these ingredients in an integrated manner with the metabolic responses, intestinal health, and productive performance of fish. This limits the understanding of their nutritional effects. This study evaluated the apparent digestibility of nutrients and energy from starch-rich practical diets and their effects on growth performance, intestinal fold histomorphometry, and metabolic and physiological profiles of tambaqui (*Colossoma macropromum*). One hundred and ninety-two tambaqui juveniles (131.63 ± 1.94 g) were fed six experimental, pelleted diets formulated with corn (CO), cornstarch (CS), sorghum (SO), wheat bran (WB), rice bran (RB), or broken rice (BR). The apparent digestibility coefficients of dry matter (ADC_{DM}), organic matter (ADC_{OM}), ether extract (ADC_{EE}), crude protein (ADC_{CP}), gross energy (ADC_{GE}), and starch (ADC_{ST}) were analyzed. ADC_{DM} (31.8%–71.9%), ADC_{OM} (44.5%–76.6%), and ADC_{GE} (48.0%–72.6%) varied among the diets but followed a similar pattern: values for the diets with CO (63.3%, 71.7%, and 72.6%, respectively), CS (71.9%, 76.6%, and 79.2%, respectively), and broken bran (65.6%, 72.4%, and 72.5%, respectively) were higher than those for the diets with WB (41.0%, 52.2%, and 53.8%, respectively) and RB (31.8%, 44.5%, and 48.0%, respectively). ADC_{CP} and ADC_{EE} were $> 70\%$ in all diets, except that with WB. ADC_{ST} was $> 90\%$ for all diets. Plasma glucose and serum cholesterol concentrations were similar across treatments. Diets including WB and RB resulted in less-developed intestinal folds, which led to lower digestibility and, consequently, reduced growth. In contrast, diets formulated with SO, BR, or CS proved to be viable alternatives to the CO-based diet, promoting comparable nutrient and energy digestibility. Tambaqui exhibited metabolic adaptation to the physicochemical properties of these formulations, maintaining productive performance. However, the inclusion of WB and RB should be limited because of the high fiber content, which can compromise intestinal health and nutrient utilization efficiency.

Jeisson Emerson Casemiro Ferrari and Maria Karolaine Moriman Delgado contributed equally to this manuscript.

1 | Introduction

The substantial contribution of nutrition to the total cost of aquaculture production has encouraged nutritionists and researchers to seek economical and sustainable ingredients for fish-feed formulations. Beyond economic concerns, the availability of essential protein and fatty acid sources such as fish meal and fish oil is increasingly limited due to pressure on wild fish stocks and long-term sustainability risks [1, 2]. These challenges have intensified the search for plant-based alternatives that are not only cost-effective but also compatible with the principles of responsible aquaculture [3, 4]. As a result, the increased inclusion of carbohydrate-rich plant ingredients into aquafeeds has become increasingly inevitable [5–8], since modern practical diets are formulated on the premise that fish do not require specific ingredients, but rather a balanced supply of nutrients that meet their dietary requirements, which can be achieved through different combinations of raw materials [9].

Cereal grains and their byproducts, such as sorghum (SO), broken rice (BR), rice bran (RB), and wheat bran (WB), stand out because of their availability and lower cost than ingredients of animal origin [10–13]. However, plant-based ingredients contain antinutritional factors (ANFs), including nonstarch polysaccharides (NSPs), phytates, tannins, and protease inhibitors, which can reduce feed palatability, damage intestinal structure, and compromise nutrient absorption and animal performance [14, 15]. The degree of influence of ANF depends, among other determinants, on the processing technologies used in feed manufacturing that are capable of reducing and/or neutralizing these compounds [16] and on the anatomical and physiological characteristics of the animal's digestive system that confer the ability to digest and metabolize nutrients of plant origin [17].

Tambaqui (*Colossoma macropomum*), an omnivorous species that plays an important ecological role in seed dispersal across the floodplain forests of the Amazon Basin, possesses a natural ability to utilize vegetable-origin feedstuffs, making it an ideal candidate for studies involving the inclusion of such inputs in aquafeed formulations [18, 19]. *Colossoma macropromum* is one of the main species cultivated in South and Central America [20] and its production has expanded to Asian countries such as Thailand, China, Vietnam, and Malaysia [21]. In Brazil, tambaqui is among the most farmed native species, and in 2023, it was the second-most exported species [22]. The growing relevance of tambaqui in aquaculture can be explained by their favorable characteristics for farming, such as high growth rate and adaptability to artificial feeds and different production systems, in addition to the ease of obtaining juveniles and high acceptance by the consumer market [23].

Despite its ecological, economic, and social importance, the nutritional management of farmed tambaqui is based on generalist feeds, which disregard the specific nutritional requirements and physiological aspects of the species [24]. This limitation contrasts with the NRC [25] guidelines, which emphasize the importance of quantitatively evaluating all dietary components in a detailed and integrative manner, especially given the diversity of available plant ingredients and the high variability in their chemical composition. Most studies on *C. macropomum* have focused on the apparent digestibility of ingredients [26–29], whereas investigations of additional variables, such as metabolic and hematological responses and body composition, are limited to a small number of ingredients, which restricts a more comprehensive view of the effects of different raw materials [30–33].

Among the variables still overlooked in studies on this species, intestinal structure stands out, as the intestine functions as a key organ at the interface between diet and physiology, playing a critical role in both nutrient absorption and the maintenance of the organism's functional integrity [34, 35]. Accordingly, the inclusion of plant-based ingredients in aquafeeds has been consistently linked to structural alterations in the intestinal mucosa, primarily due to the presence of antinutritional compounds with inflammatory properties [36]. In Nile tilapia (*Oreochromis niloticus*), fully plant-based diets induce severe epithelial damage and suppress intestinal cell proliferation [37]. Similarly, in herbivorous species such as gibel carp (*Carassius auratus gibelio*) and grass carp (*Ctenopharyngodon idella*), prolonged exposure to such formulations has led to intestinal inflammation [38, 39]. In this context, histological assessment focused on the morphometric analysis of intestinal folds enables the detection of structural alterations such as fold atrophy and compromised epithelial integrity, as demonstrated in various fish species [40–46]. Therefore, this method serves as a reliable approach to estimate the cumulative impact of dietary ingredients on fish intestinal health [47, 48].

Previous studies on tambaqui nutrition, such as the determination of the nutritional requirements for proteins and amino acids [49–51], carbohydrate tolerance [18], and nutrient digestion and metabolism [52–54], are valuable tools for elucidating the nutritional and physiological characteristics of the species. However, to enhance the applicability of scientific findings to commercial aquaculture, it is essential to advance toward experimental approaches that incorporate different feedstuffs within the context of practical diets [55–57]. This strategy provides a more realistic representation of the challenges faced by nutritionists in adjusting feed formulations to the inherent variability of available plant ingredients. Therefore, this study evaluated, in an integrated manner, the apparent digestibility of practical diets formulated with energy ingredients of plant origin and their effects on intestinal histomorphometry, blood metabolites, and productive performance of juvenile tambaqui.

2 | Materials and Methods

All procedures were conducted according to the ethical principles of animal experimentation adopted by the Ethics Committee on Animal Use (CEUA/UNESP), under Protocol Number 09/2021.

2.1 | Experimental Ingredients and Diets

Experimental diets were formulated to meet the nutritional requirements of the species according to Buzollo et al. [58], with fish meal, soybean meal, soy protein concentrate, and corn (CO) as the main ingredients. Six energy ingredients were evaluated: CO, SO, cornstarch (CS), WB, RB, and BR. The experimental diets were formulated to contain 0.5% chromium oxide III (Cr_2O_3), which was used as an inert digestibility marker [59] (Table 1). All the dietary ingredients were ground, mixed, and granulated using a pelleting machine (Model PBM081, Beccaro Equipamentos Industriais Ltd, Rio Claro, SP, Brazil). All diets were pelleted under identical processing conditions, with the same temperature and mechanical

TABLE 1 | Composition and nutrient levels of experimental diets (dry basis).

Ingredients (%)	Experimental diets					
	CO	SO	CS	WB	RB	BR
Fish meal	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal	21.00	21.00	21.00	21.00	21.00	21.00
Soybean protein concentrate	15.90	15.50	19.40	13.40	14.50	16.70
Corn	38.50	8.50	8.50	8.50	8.50	8.50
Sorghum	—	30.00	—	—	—	—
Cornstarch	—	—	30.00	—	—	—
Wheat bran	—	—	—	30.00	—	—
Rice bran	—	—	—	—	30.00	—
Broken rice	—	—	—	—	—	30.00
Soybean oil	3.50	3.50	3.50	3.50	3.50	3.50
Mineral and vitamin supplement[a]	0.80	0.80	0.80	0.80	0.80	0.80
Dicalcium phosphate	0.75	0.75	0.75	0.75	0.75	0.75
Vitamin C	0.02	0.02	0.02	0.02	0.02	0.02
Chromium oxide	0.50	0.50	0.50	0.50	0.50	0.50
Kaolin	4.03	4.43	0.53	6.53	5.43	3.23
Composition (%)[b]						
Dry matter	94.22	94.68	94.69	94.81	94.56	94.78
Crude protein	30.90	34.25	32.17	30.68	33.09	32.56
Ether extract	6.36	8.03	6.23	7.51	10.16	5.84
Mineral matter	11.91	13.39	9.31	15.67	17.11	11.13
Calcium	1.94	2.19	2.11	2.03	2.18	2.21
Phosphorus	0.92	1.18	0.98	1.21	1.55	1.03
Starch	47.54	44.53	64.93	19.74	28.19	53.39
Neutral detergent fiber	4.17	4.03	3.46	7.50	6.00	3.36
Acid detergent fiber	4.67	4.51	4.27	7.03	6.96	5.33
Gross energy (kcal kg^{-1})	4.043	4.125	4.174	4.078	4.138	4.091
IFN[c]	4-02-935	4-04-383	4-02-889	4-05-190	4-03-930	4-03-932

Abbreviations: BR, broken rice; CO, corn; CS, cornstarch; RB, rice bran; SO, sorghum; WB, wheat bran.

[a]Composition of vitamin and mineral supplement (Premix) for omnivorous fish: humidity = 5%; dry matter = 95%; Mn = 2000 mg; Fe = 7500 mg; Zn = 7500 mg; Cu = 1000 mg; Co = 30 mg; Se = 70 mg; I = 250 mg; K = 2000 mg; Mg = 600 mg; vitamin A = 2,000,000 IU; vitamin D_3 = 600,000 IU; vitamin K_3 = 700 mg; biotin = 50 mg; folic acid = 250 mg; choline = 80,000 mg; vitamin B_1 = 2000 mg; vitamin B_{12} = 10,000 mcg; vitamin B_2 = 4000 mg; vitamin B_6 = 5000 mg; vitamin E = 15,000 UI; pantothenic acid = 5000 mg; nicotinic acid = 10,000 mg; vitamin C = 80,000 mg; BHT = 20,000 mg; inositol = 4000 mg.

[b]Composition calculated from the composition of the ingredients analyzed.

[c]International Food Number referenced by the NRC [25].

pressure applied across treatments. The pellets were dried in an air-circulating oven (40°C) for 24 h and stored in plastic containers under refrigeration at 5°C until further use.

2.2 | Digestibility Trial

The digestibility of the diets was determined using an indirect method of collecting feces. The trial lasted 15 days, with the first 10 days used for the acclimation and adaptation of fish to experimental diets, and the remaining 5 days dedicated to feces collection for digestibility analyses. The experimental setup consisted of two systems of tanks connected to a recirculating aquaculture system. The first system included 24 polyethylene tanks (300 L) used exclusively for feeding, to prevent fecal contamination by feed residues. The second consisted of six conical bottom tanks (80 L) with a decantation-designed feces collection system composed of collector tubes immersed in ice to prevent the degradation of feces, according to the method of Tanaka et al. [12]. Throughout the experimental period, both feeding and digestibility tanks were kept clean, avoiding the accumulation of feed residues and possible fecal contamination.

One hundred and ninety-two fish averaging 131.63 ± 1.94 g were distributed in the feeding tanks in quadruplicate groups for each experimental diet. This setup resulted in eight fish per tank. One hour after the last feeding, one group from each dietary treatment (six total) was randomly selected and transferred to the conical bottom tanks, where they remained for 8 h for feces collection. This procedure was repeated daily until all experimental units had been evaluated, ensuring that fecal samples were obtained from every replicate of each dietary treatment. The feces decanted in the tubes were collected, transferred to Petri dishes, separated from possible scales, and subsequently dried in an oven with forced ventilation at 40°C for 24 h. The fecal samples were then ground, homogenized using a mortar and pestle, and conditioned for further nutritional analyses.

Analyses of the chemical compositions of diets and feces were performed according to methodologies described by the Association of Official Analytical Chemists [60]. In brief, dry matter (DM) was obtained by drying in an oven at 105°C for 24 h; organic matter (OM) was calculated as the DM minus the mineral matter; the ether extract (EE) was determined after extraction with petroleum ether in a Soxhlet-type heating block (Tecnal, TE-044, Piracicaba, SP, Brazil), followed by removal by evaporation or distillation of the solvent used; the crude protein (CP) by the digestion method with sulfuric acid, distillation with sodium hydroxide in a Kjeldahl distiller (Tecnal, MA-036, Piracicaba, SP, Brazil) and titration with hydrochloric acid and; the gross energy (GE) was determined by direct combustion in a bomb calorimeter with oxygen (IKA/C2000 Basic, IKA Works, Inc. North Chase Pkwy SE, Wilmington, USA). Starch was determined using the amylase and glucose-oxidase method [61], and chromium was quantified by atomic absorption spectrophotometry (Thermo Scientific, Waltham, MA, USA) after nitric perchloric digestion, according to the method of Furukawa and Tsukahara [62].

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of the experimental diets were analyzed according to Van Soest et al. [63], using a Marconi MA-444/CI Fiber Digestor (Marconi LTDA, Piracicaba, SP, Brazil).

After performing a quantitative analysis of Cr_2O_3 and nutrients in the feces and experimental diets, the apparent digestibility coefficients (ADCs) of the nutrients (DM, OM, EE, CP, and ST) and the energy of the different treatments were calculated according to the methodology described by the NRC [25] as:

$$\text{ADC nutrients or energy (\%)} = 100 - 100 \times \left(\frac{\%\, Cr_2O_3\ \text{diet}}{\%\, Cr_2O_3\ \text{feces}}\right) \times \left(\frac{\%\ \text{nutrients or energy feces}}{\%\ \text{nutrients or energy diet}}\right). \quad (1)$$

2.3 | Growth Performance Trial

The tambaqui juveniles ($n = 192$; initial weight $= 131.63 \pm 1.94$ g) were randomly distributed into 24 polyethylene tanks with a capacity of 300 L, organized in a water recirculation system with mechanical–biological filtration, additional aeration, and controlled temperature. The stocking density corresponded to eight fish per tank, averaging approximately 3.51 kg m^{-3}. The fish were acclimatized for 10 days, during which they were fed a commercial diet (280 g kg^{-1} CP; NutriPiscis Presence Animal Nutrition, Santa Rosa, Brazil). After this period, the animals were fasted for 24 h and stunned (clove oil, 100 mg L^{-1}) to perform the initial biometry.

The experimental diets were randomly distributed to quadruplicate groups of fish, following a completely randomized design consisting of six treatments and four replicates. The fish were fed four times a day for 57 days until apparent visual satiation. The dissolved oxygen concentration (6.80 ± 0.29 mg L^{-1}) and temperature (27.54 ± 0.30°C) remained within the ranges considered ideal for the species [64]. The laboratory lighting was maintained using fluorescent lamps to provide a photoperiod of 12 h light and 12 h darkness.

At the end of the experimental period, the previously anesthetized (clove oil, 100 mg L^{-1}) fish were subjected to biometric handling to measure their weights. The biometric data obtained and the amount of feed provided were used to calculate the following productivity performance parameters:

$$\text{Weight gain (WG, g)} = \text{Final weight} - \text{initial weight}. \quad (2)$$

$$\text{Feed intake (FI, g/day)} = \text{Amount of feed provided} - \text{the uneaten feed}. \quad (3)$$

$$\text{Protein efficiency rate (PER, \%)} = \frac{\text{Weight gain}}{\text{Feed intake} \times \text{CP\% of diet}} \times 100. \quad (4)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Body weight gain}}. \quad (5)$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) = \left(\frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Days of experiment}}\right) \times 100. \quad (6)$$

2.4 | Evaluation of the Metabolic Profile

At the end of the performance trial and after fasting for 24 h, three fish per tank (12 fish per treatment) were anesthetized with clove oil (100 mg L^{-1}) for blood collection by caudal vessel puncture with disposable syringes and needles. Blood aliquots were divided into microtubes without and with anticoagulant (EDTA with potassium fluoride; Bioclin-Quibasa Basic Chemistry, Belo Horizonte-MG, Brazil) and subsequently centrifuged at $827 \times g$ (4°C; 10 min) to obtain serum and plasma, respectively. Cholesterol (enzymatic colorimetric method, Bioclin-Quibasa Basic Chemistry Ltd., Belo Horizonte-MG, Brazil), serum triglycerides (enzymatic colorimetric method, Labtest Diagnostic SA, Lagoa Santa-MG, Brazil), total serum protein (biuret method; Reinhold, 1953, Labtest Diagnostic SA, Lagoa Santa-MG, Brazil), and plasma glucose (GOD-Trinder method, Bioclin-Quibasa Basic Chemistry, Belo Horizonte-MG, Brazil) was quantified. Analyses were performed in duplicate, and blood metabolites were measured using a spectrophotometer (UV-Vis, Thermo Fisher Scientific Inc., Evolution 60S, Madison, USA).

2.5 | Somatic Indices and Histological Analyses

After blood collection, the fish were sacrificed by deep anesthesia with clove oil (300 mg L^{-1}) and then underwent laparotomy to remove the visceral fat and liver, which were weighed to calculate the mesenteric fat index (MFI, %) and hepatosomatic index (HSI, %), respectively, according to the following formula:

$$\text{MFI and HSI} = 100 \times \left(\frac{\text{tissue weight}}{\text{live weight}}\right). \quad (7)$$

Fragments (2 cm) were collected from the proximal region of the intestine, dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques [65]. The sections were stained with hematoxylin and eosin [66]. The prepared slides were photodocumented using an Axio Scope A1 photomicroscope (Carl Zeiss Microscopy, LLC, White Plains, NY, USA), and morphometric measurements were performed using ImageJ software (Version 1.49f, National Institutes of Health, USA).

The height and width of 60 intestinal folds were measured for each treatment. The height was measured from the base to the apex of the intestinal fold, and the width was obtained by measuring the median transverse dimensions of each fold (Figure 1).

2.6 | Statistical Analysis

Data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Bartlett test). Upon confirmation, the data were subjected to analysis of variance (one-way ANOVA), followed by Tukey's multiple comparison test, at a significance of $p < 0.05$. Results are expressed as mean ± standard deviation. All analyses were performed using the ExpDes.pt package [67] in R software, version 4.2.1.

3 | Results

3.1 | Digestibility Coefficient

ADCs values are presented in Figure 2. The practical diets containing CO (63.3%), CS (71.9%), BR (65.6%), and SO (54.9%) showed higher DM ADC (ADC_{DM}) than those including WB (41%) and RB (31.8%), with the latter being similar to each other.

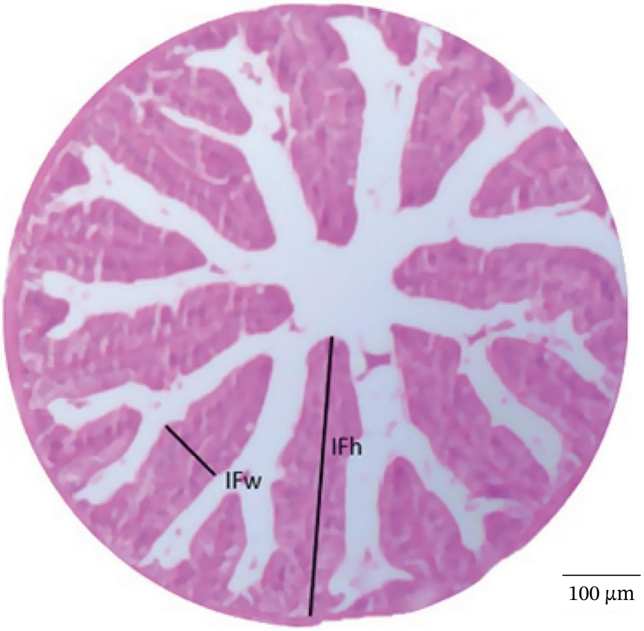

FIGURE 1 | Histological section of the proximal intestine of juvenile tambaqui showing the width of the intestinal fold (IFw) and the height of the fold (IFh). Scale bars 100 µm (10x).

The ADC_{DM} of the SO diet was similar to that of the CO diet, but lower than that of the CS and BR diets. The OM digestibility coefficients (ADC_{OM}) were similar between CO (71.7%), SO (64.5%), CS (76.6%), and BR (72.4%) diets, and higher than those of WB (52.2%) and RB (44.5%). The CP digestibility coefficients (ADC_{CP}) of formulated with CO, SO, CS, and BR were similar and exceeded 75%. The RB diet had the lowest ADC_{CP} (66.6%), but did not differ from the WB diet (72.8%). Furthermore, protein from the CO diet was approximately 10% more digestible than that from the WB diet.

For the apparent digestibility of GE (ADC_{GE}), the same pattern was observed for the average ADC_{DM}. Higher ADC_{GE} values were observed in diets formulated with CO (72.6%), CS (79.2%), and BR (72.5%), whereas lower values were obtained with SO (66.7%), WB (53.8%), and RB (48.0%) diets. The EE portions of CO, SO, WB, and BR diets were highly digestible by juvenile tambaquis, with apparent digestibility coefficient values of EE (ADC_{EE}) > 75%. However, RB diet had a lower ADC_{EE} (69.9%) compared with the CO (87.9%), SO (89.5%), and CS (86.1%) diets. Starch digestibility coefficients (ADC_{ST}) did not differ between treatments and were > 90% across all diets.

3.2 | Productive Performance

No differences were observed among the evaluated diets in terms of protein efficiency rate (PER), feed intake (FI), feed conversion ratio (FCR), or specific growth rate (SGR; $p > 0.05$; Table 2). However, the WB-containing diet produced a lower final weight and weight gain for tambaquis compared to fish that received CS diet, but there were no differences among the other diets.

3.3 | Intestinal Morphometry

The intestinal fold height of fish fed diets containing CO, SO, and CS was greater than that of fish fed RB diet ($p < 0.05$), with no significant difference between fish fed WB and BR diets (Table 3). The widths of the intestinal folds of tambaquis fed WB and RB diets were smaller than those of the BR group and comparable to those of fish fed CO, SO, and CS diets.

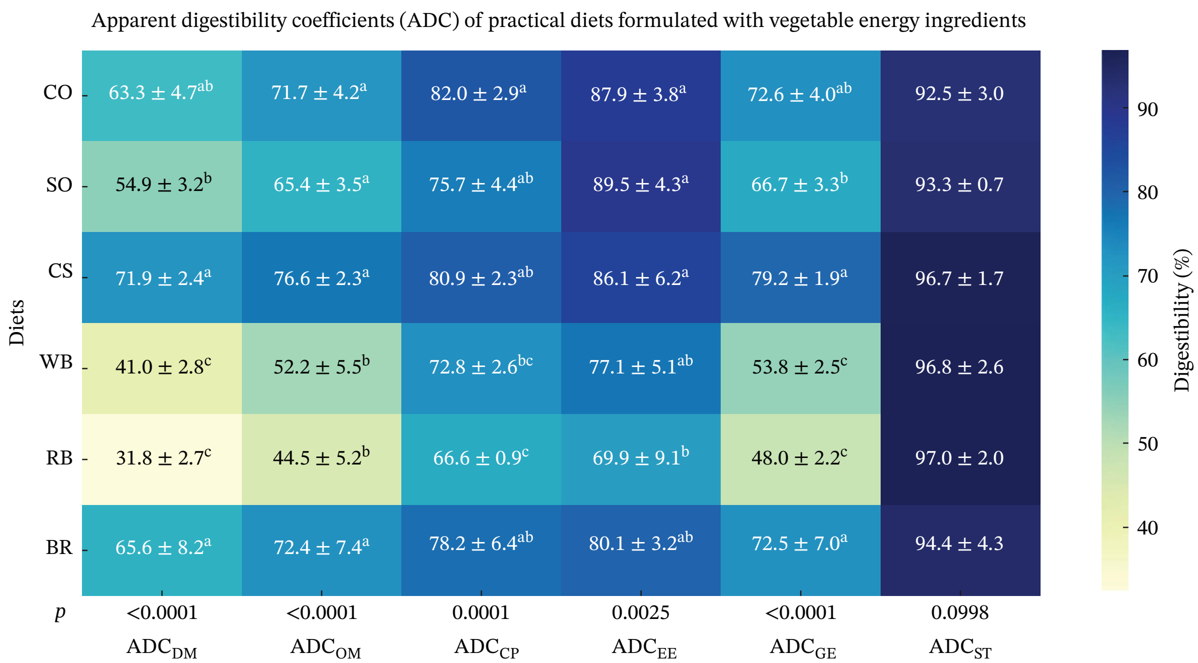

FIGURE 2 | Apparent digestibility coefficients (ADC, %) of practical diets formulated with vegetable energy ingredients for juvenile tambaquis. Data represent mean ± standard error of four replicates ($n = 4$). Different superscript letters in the same column indicate that the means differed from each other according to Tukey's test, with a probability of 5% ($p < 0.05$). Diet codes: CO, corn; SO, sorghum; CS, cornstarch; WB, wheat bran; RB, rice bran; BR, broken rice. Apparent digestibility coefficients (ADC): DM, dry matter; OM, organic matter; CP, crude protein; GE, gross energy; EE, ether extract; ST, starch.

TABLE 2 | Productive performance of juvenile tambaquis fed practical diets formulated of vegetable energy ingredients for 57 days.

Diets	Variables					
	FW (g)	WG (g)	PER (%)	SGR (% day^{-1})	FI (g)	FCR
CO	203.8 ± 5.3^{ab}	71.7 ± 4.8^{ab}	1.9 ± 0.1	0.76 ± 0.0	981.8 ± 16.8	1.7 ± 0.1
SO	201.7 ± 4.0^{ab}	70.7 ± 3.3^{ab}	1.9 ± 0.1	0.76 ± 0.0	979.6 ± 18.5	1.7 ± 0.1
CS	206.9 ± 11.7^{a}	75.4 ± 11.5^{a}	2.0 ± 0.3	0.79 ± 0.0	977.5 ± 29.5	1.6 ± 0.3
WB	190.1 ± 5.6^{b}	58.7 ± 3.9^{b}	1.6 ± 0.1	0.65 ± 0.0	956.5 ± 31.4	2.0 ± 0.2
RB	196.6 ± 3.7^{ab}	66.4 ± 3.9^{ab}	1.7 ± 0.2	0.72 ± 0.0	987.3 ± 33.2	1.9 ± 0.2
BR	203.7 ± 6.2^{ab}	71.4 ± 6.3^{ab}	1.9 ± 0.2	0.75 ± 0.0	984.8 ± 26.2	1.7 ± 0.2
p	0.02	0.04	0.14	0.07	0.64	0.11

Note: Data represents mean ± standard error of four replicates ($n = 4$). Different superscript letters in the same column indicate that the means differed from each other according to Tukey's test, with a probability of 5% ($p < 0.05$). Diet codes: CO, corn; SO, sorghum; CS, cornstarch; WB, wheat bran; RB, rice bran; BR, broken rice. Variables: FW, final weight; WG, weight gain; PER, protein efficiency rate; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio. FW and WG reflect overall growth performance, SGR indicates growth rate over time, PER represents protein utilization efficiency, and FCR expresses feed use efficiency.

3.4 | Metabolic Profile and Somatic Indices

No differences ($p > 0.05$) were observed in plasma glucose or serum total cholesterol contents. However, the serum triglyceride, total protein concentrations, HSI, and MFI showed marked variations among diets (Figure 3). Fish fed WB and RB diets had lower triglyceride concentrations than those in the CS diet. In contrast, fish fed the CO, SO, CS, and BR diets showed similar serum triglyceride contents. Total protein content was the highest in the RB diet and lowest in the SO diet. The HSI was lower in fish that consumed the CO diet (1.28%) than in those that consumed SO (1.45%), CS (1.49%), WB (1.44%), or BR (1.53%) diets, with no differences from the animals fed RB (1.40%). Furthermore, the fish in the RB diet had a lower HSI than those in the BR diet. MFI was higher in fish that received SO (0.73%), CS (0.90%), and BR (0.75%) diets and lower in animals fed WB (0.44%) and RB (0.59%) diets. The fish fed CO had an MFI comparable to that of the groups fed SO, WB, and RB diets, but the MFI was lower than that of the animals fed the CS diet.

TABLE 3 | Height and width of the intestinal fold of juvenile tambaquis fed practical diets formulated different vegetable energy ingredients for 57 days.

Diets	Variables	
	Height (μm)	Width (μm)
CO	232.5 ± 10.3^{a}	48.4 ± 3.0^{ab}
SO	228.9 ± 15.5^{a}	50.3 ± 2.5^{ab}
CS	227.9 ± 20.3^{a}	53.9 ± 9.0^{ab}
WB	208.4 ± 22.3^{ab}	45.3 ± 3.9^{b}
RB	173.8 ± 21.8^{b}	45.1 ± 5.7^{b}
BR	208.5 ± 15.1^{ab}	57.4 ± 3.7^{a}
p	0.002	0.02

Note: Data represents mean ± standard error ($n = 60$). Different superscript letters in the same column indicate that the means differed from each other according to Tukey's test ($p < 0.05$). Diet codes: CO, corn; SO, sorghum; CS, cornstarch; BR, broken rice; WB, wheat bran; RB, rice bran.

4 | Discussion

Digestibility studies are essential to determine the bioavailability of nutrients and energy utilized by fish, support the formulation of nutritionally-effective, cost-efficient, and environmentally-sustainable diets [68]. In the current study, the ADCs for DM, OM, and GE varied among the diets tested; however, the statistical differences followed a consistent pattern. Diets containing WB and RB had the lowest digestibility values for DM (41.0% and 31.8%, respectively), OM (52.2% and 44.5%, respectively), and GE (53.8% and 48%, respectively). These results are mainly attributed to differences in the structural composition of the ingredients, which directly affect their physicochemical properties and digestibility. In the present study, the diets containing WB and RB exhibited the highest levels of NDF (7.5% and 6.0%, respectively) and ADF (7.0% and 6.9%, respectively), whereas the diets formulated with CO, SO, CS, and BR showed lower NDF (3.4%–4.2%) and ADF (4.3%–5.3%) contents. CO, SO, and BR are derived from whole grains and therefore have a higher proportion of endosperm, rich in starch and easily digestible components, while WB and RB, which are by-products of grain processing, are composed mainly of outer layers rich in cellulose, hemicellulose, pectin, and lignin [69], components that correspond directly to NDF and ADF values. Such fibrous fractions are not degraded by endogenous enzymes in animals and, together with NSPs, act as physical barriers to nutrient bioavailability by increasing the intestinal transit time or digesta viscosity. This effect impairs enzyme–substrate interactions, resulting in incomplete digestion and absorption [70, 71].

Consistent with our results, previous studies have also observed lower assimilation of DM and GE in the WB of tambaqui (86.5 g), pirapitinga (*Piaractus brachypomus*, 220 g), and the tambacu hybrid (*C. macropomum* × *Piaractus mesopotamicus*, 17 g) [26, 72, 73]. Additionally, our study found lower digestibility values for DM and GE in the SO-containing diet than in the CS diet. This result is consistent with previous reports that identified a lower ADC_{DM} for SO than for CS [17]. It is likely that the tannins present in SO, known as anti-nutritional phenolic compounds, form complexes with proteins, polysaccharides, fatty acids, and nucleic acids [74], thereby reducing the digestibility of DM, and consequently, energy utilization.

Protein utilization can be influenced by factors such as intestinal transit time, protease concentration and activity, and the presence

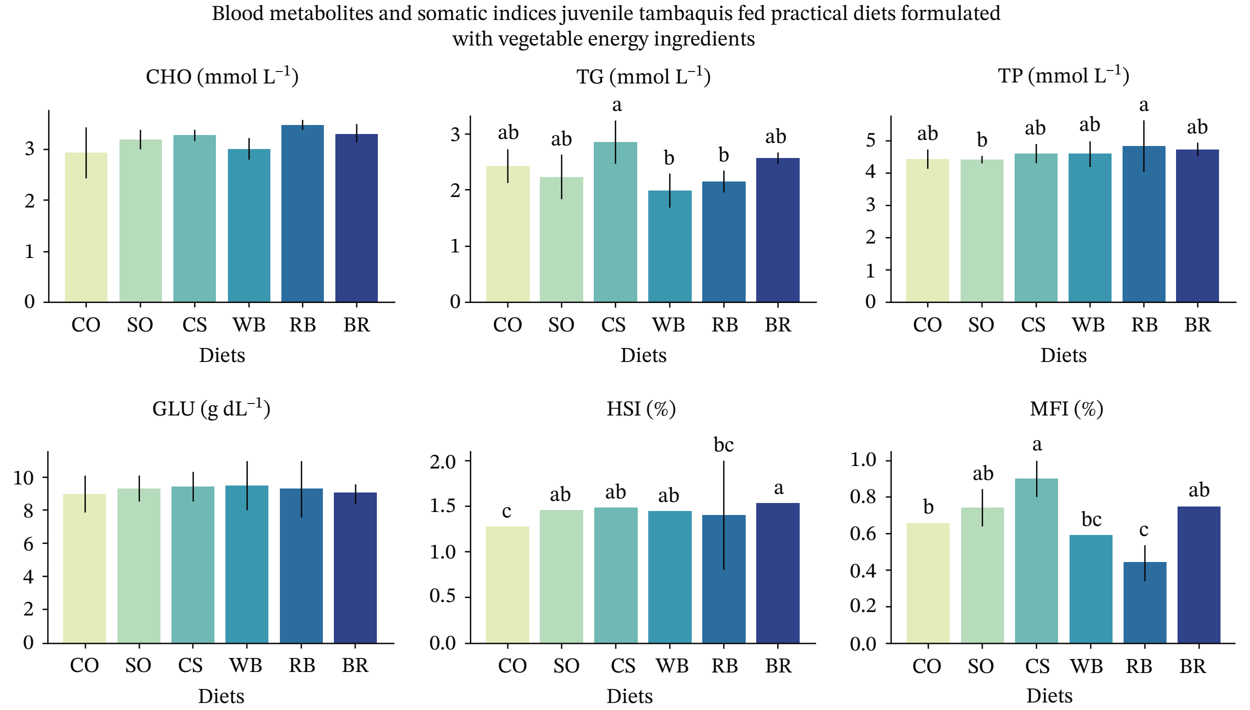

FIGURE 3 | Mean values (±standard error) of blood metabolites and somatic indices of juvenile tambaquis ($n = 4$) after 57 days of feeding practical diets formulated with different vegetable energy ingredients. Different superscript letters indicate significant differences among treatments (Tukey's test, $p < 0.05$). CHO, cholesterol; GLU, glucose; HSI, hepatosomatic index; MFI, mesenteric fat index; TG, triglycerides; TP, total protein. HSI provides an indirect estimate of hepatic energy reserves, whereas MFI reflects visceral lipid deposition. Diet codes: CO, corn; SO, sorghum; CS, cornstarch; WB, wheat bran; RB, rice bran; BR, broken rice.

of antinutritional components [75]. In the present study, the utilization of the protein fraction exceeded 75% for CO, SO, CS, and BR diets, demonstrating the potential of these ingredients in formulating diets for juvenile tambaquis. However, the RB diet showed a lower protein digestibility than the other diets, except for WB-containing diet, which had similar values. The CP from the WB diet was approximately 10% less digested than that in the CO diet. Consistent with our results, other studies have reported lower protein digestibility of WB compared to CO in juvenile tilapia [76] and tambaqui [26]. The reduced protein utilization observed in RB and WB diets is associated with their higher structural fiber contents [77].

Abimorad and Carneiro [59] reported high protein digestibility of WB and RB in pacu (250 g). Their study used manual extrusion for fecal collection, whereas the current study used an indirect sedimentation method. Some authors have indicated that digestibility values obtained using manual extrusion tend to be higher because of the higher concentration of chromium oxide in the accumulated and expelled feces in one time caused by the pressure exerted on the fish abdomen [78, 79].

The apparent digestibility of the EE (ADC_{EE}) was lower for diets containing RB (69.9%) than for diets formulated with CO (87.9%), SO (89.5%), and CS (86.1%). This reduced digestibility presumably resulted from the high crude fiber content of RB, which limits enzyme access to fats and hinders lipid digestion [18]. ADC values above 90% for SO, WB, and BR have been reported in juvenile tambaqui [58]. These discrepancies may be attributed to the processing methods used. In the present study, pelleted diets were employed to minimize processing-related variation, whereas other studies used extruded diets. The extrusion process combines moisture, pressure, temperature, and mechanical shear, leading to protein denaturation, starch gelatinization, and lipid cell rupture, which makes nutrients more available for digestion [80, 81], but may also amplify or mask the nutritional differences among ingredients in the diets tested [82].

Starch is the main energy reserve of plants, consisting of 20%–30% amylose (linear structure) and 70%–80% amylopectin (branched structure). The shape and size of starch granules, along with the amylose-to-amylopectin ratio, vary among plant sources, influencing their resistance to enzymatic digestion, as verified in a previous study on *Piaractus mesopotamicus* [83]. However, tambaqui utilized more than 90% of the starch from all diets tested, a result comparable to that of juvenile tilapia fed diets containing different starch sources [84]. The long intestinal anatomical characteristics of the omnivorous eating habit, in addition to the adequate activity of carbohydrases, appear to be sufficient to overcome the influence of dietary fiber and ensues efficient digestion of starch of different chemical and structural characteristics.

Growth and feeding efficiency are practical and sensitive indicators of adaptation to specific diets or ingredients. In this study, tambaqui-fed diets containing WB exhibited reduced final weight and weight gain compared to those receiving the diet formulated with CO, which can be attributed to the lower digestibility of DM, OM, and GE. Juvenile tilapia fed WB without exogenous enzyme supplementation demonstrated results similar to those in a previous study [11]. Diets with a high WB content generally have reduced digestible energy and total apparent digestibility of nutrients for growing animals [85]. Despite these findings, no statistical differences were found in PER, FI, FCR, and SGR among treatments. The ability of tambaquis to adapt to the chemical and physical attributes of various ingredients, owing to their omnivorous and frugivorous feeding habits, explains the lack of substantial differences in growth and feed utilization during the short study period.

Changes in the intestinal morphology are closely related to the digestive and absorptive functions of fish. The tambaqui fed diets based on CO, SO, and CS had higher intestinal fold heights than the animals fed diet containing RB, and the widths of the intestinal folds of the fish fed diets including WB and RB were smaller than those of the fish fed diet formulated with BR. The intestinal morphological alterations are consistent with intestinal responses commonly reported in fish fed diets containing plant-derived antinutritional factors. Several studies have demonstrated that such compounds stimulate the upregulation of pro-inflammatory cytokines such as IL-1β and TNF-α within the intestinal microenvironment, promoting the infiltration of inflammatory cells (T lymphocytes, mast cells, macrophages, and neutrophils) which contributes to lamina propria thickening and the shortening and fusion of intestinal folds [86–91].

This mechanism was demonstrated by Liang et al. [92], who reported increased TNF-α expression, along with a significant reduction in mucosal thickness and fold height in juvenile largemouth bass (*Micropterus salmoides*) fed high levels of soybean protein concentrate. Similarly, Willora et al. [93] observed thickening of the lamina propria and decreased villus height in juvenile lumpfish (*Cyclopterus lumpus*) fed diets containing varying levels of fish meal replacement with soybean and pea protein concentrates. In addition, catfish (*Rhamdia quelen*) fed crambe bran (*Crambe abyssinica*) also showed lower fold heights because of the antinutritional factors present in this ingredient [94].

Taken together, these inflammatory and structural processes may account for the lower apparent digestibility coefficients observed in diets containing WB and RB, as reductions in intestinal fold height and thickness restrict the effective absorptive interface [95]. Conversely, the preservation of intestinal fold architecture in fish fed diets containing CO, SO, CS, and BR supports more efficient nutrient utilization and, consequently, improved productive responses [96].

Dietary composition can alter blood metabolite concentrations due to biochemical adjustments in metabolism. Nutrient utilization can be partially assessed using the HSI, which indirectly reflects glycogen and/or lipid deposition in the liver, and the MFI, which indicates visceral fat storage [97]. In this present study, the test diets showed modest variations in protein, lipid, and starch levels, which is characteristic of nutritional investigations employing practical formulations with the inclusion of plant-based ingredients with distinct nutritional profiles [30, 31, 98, 99]. Given the sensitivity of lipid metabolism to dietary macronutrient balance, part of the variation observed in somatic indices may be related to differences in overall diet composition. Therefore, the metabolic responses should be interpreted in an integrated manner alongside data on digestibility, intestinal morphometry, and growth performance.

Fish fed the CS diet had higher serum triglyceride levels and MFI than those fed WB and RB diets. Although the ADC values for starch did not vary between the tested treatments, the formulation with CS had a higher percentage of starch (64.9%) than the WB (19.7%) and RB (28%) diets; therefore, the amount of carbohydrates digested and absorbed was greater in fish fed the diet containing CS, reflecting increased triglyceride levels, indicative of lipogenesis and storage in visceral adipose tissue. Research carried out on juvenile tambaqui [52] and pacu [100] fed diets containing 30%–50% starch found elevated serum triglyceride levels and increased fat deposition in the liver and viscera, respectively. Furthermore, the lower triglyceride levels and MFI observed in fish fed WB and RB diets, and the lower HSI of fish fed RB diet compared to BR can be attributed to the lower ADC of different nutrients and energy.

Controlling lipid synthesis and deposition in fish is important to guarantee balanced energy metabolism. In this study, fish fed the CO-based diet had a lower HSI than those treated diets formulated with CS, SO, WB, and BR; however, the MFI was similar among treatments with SO, WB, and BR. The results of the present study suggest that the triglycerides in fish receiving the CO diet are preferentially stored as visceral fat. Lipid deposition sites vary among species based on lifestyle, feeding behavior, and diet composition. In salmonids, for example, lipid deposition occurs primarily in the viscera and, to a lesser extent, in muscle [101]. However, to date, no studies have evaluated the effects of different diet formulations using energy ingredients on the lipid storage sites in tambaqui, which requires metabolomic approaches to understand these dynamics.

Total serum protein (TP) levels may indicate the nutritional status and feed quality of fish [102]. Changes in total protein values can indicate protein degradation and synthesis during metabolism [103]. The lower serum protein levels observed in fish fed the SO-based diet compared to the RB diet may result from metabolic adaptation to this ingredient, as most SO protein is kafirin, characterized by a rigid structural configuration in the protein matrix surrounding starch, along with low solubility and an imbalance in amino acids [104].

The ability of fish to regulate glycemic homeostasis may reveal their potential to utilize dietary carbohydrates [18, 105]. The diets formulated with plant energy ingredients evaluated in the present study did not affect glucose levels in juvenile tambaqui. Other studies have also not verified changes in glucose levels in vegetable-based energy sources of omnivorous fish [106–109]. Our findings suggest that juvenile tambaqui have a good capacity to regulate glucose absorption from the diets tested, and this is probably why the animals did not experience severe reductions in feed utilization when fed WB and RB diets.

Cholesterol reflects the nutritional status and possible dysfunction in liver lipid and lipoprotein metabolism [110]. Whereas some studies have suggested the hypocholesterolemic effects of plant ingredients in fish [111, 112], our study found no differences in serum cholesterol values in tambaquis, which is consistent with the results observed in piaparas fed the same plant ingredients [109]. This result demonstrates the metabolic capacity of omnivorous fish, specifically tambaqui, to regulate serum cholesterol levels according to the vegetable energy source available in the diet.

The integrated interpretation of the results indicates that diets formulated with CO, SO, CS, and BR maintained adequate nutrient digestibility and preserved intestinal architecture in tambaqui. In contrast, responses observed in cereal bran–based diets highlight the need for controlled inclusion of fiber-rich coproducts. From a practical perspective, diets formulated without WB or RB exhibited ADF levels up to 5.3%, which represents a reference threshold under the present experimental conditions. Accordingly, maintaining dietary ADF within this range supports maximum inclusion levels of approximately 8% for WB and RB in pelleted diets for juvenile tambaqui with a mean body weight of 131 g.

It is important to acknowledge that this study evaluated practical diets with moderate variations in macronutrient composition, particularly in starch proportions, which represents a limitation when assessing physiological responses to different carbohydrate sources. Although NDF and ADF were analyzed and provided relevant information on the structural fiber content of the evaluated ingredients, other antinutritional components commonly associated with plant-derived feedstuffs, such as soluble NSPs, tannins, and phenolic compounds, were not quantified. These constituents are known to influence digesta viscosity, enzyme–substrate interactions, and intestinal integrity; therefore, the lack of quantitative data restricts a more mechanistic interpretation of the effects observed on nutrient digestibility and gut morphology. Future studies integrating the analytical characterization of these compounds, together with graded inclusion levels of plant-derived ingredients, would allow a deeper understanding of their physiological relevance. Nevertheless, the present findings advance the understanding of integrated nutritional responses in tambaqui and provide guidance for the inclusion of alternative energy sources in practical diets, supporting more sustainable feed formulations in aquaculture.

5 | Conclusions

In this study, we demonstrated that juvenile tambaqui adapted metabolically to practical formulated diets with different plant ingredients and maintained stable glucose and cholesterol levels. Diets including BR, CS, and SO can be strategically incorporated into this specie aquaculture preserving intestinal health and nutrient and energy digestibility without compromising productive performance. However, the use of diets containing WB and RB requires caution because of their high fiber content, which can impair the growth of the intestinal epithelium and, consequently, nutritional digestibility and growth.

Author Contributions

Jeisson Emerson Casemiro Ferrari and Maria Karolaine Moriman Delgado contributed equally to this manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data are available upon request from the authors.

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