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ANIMAL SCIENCE

Effects of the replacement corn meal by whole mango meal on tambaqui (*Colossoma macropomum*) diet: Digestibility, growth performance, biochemical, and hematological responses

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Abstract: This study evaluated the digestibility of whole mango (*Mangifera indica*) meal (WMM) and determined the growth performance, intestinal enzyme activity, and metabolic and hematologic responses of tambaqui (*Colossoma macropomum*) juveniles fed diets containing different proportions of corn meal (CM) substitution by WMM. Fish fed with graded levels of WMM (0 (control), 80, 160, 240, and 320 g kg diet^{-1}), replacing part of the dietary CM. The apparent digestibility coefficients of WMM were above 96%. Diets with WMM did not affect growth performance or intestinal enzyme activity. However, they showed a positive linear effect on plasma glucose, amino acids, and albumin levels and a negative linear effect on hepatic aspartate aminotransferase activity and hepatic glycogen, plasma cholesterol, and hemoglobin levels. Increased erythrocyte values and decreased plasma triglyceride levels were verified in fish fed 80 and 160 g WMM kg diet^{-1}. In conclusion, the WMM may be a viable alternative to the tambaqui juveniles' diet, and WMM could replace up to 16% of CM without harming the growth and health of tambaqui juveniles.

Key words Apparent digestibility, corn meal, energy, erythrocytes, *Mangifera indica.*

INTRODUCTION

In practical diets of omnivorous and herbivorous fish, starch carbohydrates are commonly used to reduce the amount of protein in the food formulation (Souza et al. 2021a, b) because protein is the most expensive nutrient of these diets (Marchão et al. 2022). Therefore, a higher percentage of starch carbohydrates in the fish diet can reduce costs, saving protein as an energy source (Felix e Silva et al. 2020, Souza et al. 2021b). In addition, fish's better utilization of dietary protein can reduce the excretion of nitrogen compounds, reflecting economic and environmental gain (Yang et al. 2011).

An option to improve the use of proteins for energy purposes in fish diets is using ingredients of plant origin, as they are rich in carbohydrates (Enes et al. 2011). Among the conventional energy foods used in these diets, corn meal (CM) stands out (Felix e Silva et al. 2020, 2022). Nevertheless, international price fluctuations (da Silva et al. 2021), dependence on supply throughout the year, and difficulty transporting these products to non-producing locations can increase production costs. Thus, fruits can be an excellent alternative for diet

formulations replacing CM. Furthermore, adding fruit to the diet depends on the animal's eating habits and use at adequate levels (Souza et al. 2023).

Studies on fruit by-products are constantly growing (Tirado-Kulieva et al. 2022). Mango (*Mangífera índica* L.) is rich in carbohydrates such as starch, reducing sugars, and pectic substances (Souza et al. 2018). Mango also has high levels of probiotics, fiber, vitamin C, non-reducing sugars, polyphenols, carotenoids, and minerals in its composition (Obasa et al. 2013). It is a source of the essential amino acid profile (e.g., lysine and methionine) and contains many fatty acids (Khieokhajonkhet 2020). In mangos (*in natura*), total sugar can represent more than 50.0%, where 17.0% can be glucose, and 2.3 to 3.1% can be fructose (Bernardes-Silva et al. 2003). Fructose reaches the cells first (Enes et al. 2009) and could reduce energy costs in fish growth.

Mango also contains anti-nutritional factors (e.g., tannin, phytate, cyanogenic glycosides, oxalates, and trypsin inhibitory activity) that might affect the fish palatability (Azaza et al. 2009, Khieokhajonkhet 2020). They are substances generated by plant's secondary metabolism to protect themselves from predators (Torres-León et al. 2018). Flavonoids and phenols compounds contain hydroxyphenyl groups that, when present in animal diet, promote changes in the metabolism and, in more severe cases, health (Sreerama et al. 2010). The anti-nutritional compounds are found mainly in the mango peel (Felix e Silva et al. 2022). The type and concentrations of these compounds depend on the mango's variety, cultivation place, growth conditions, season of the year, maturity stage, and post-harvest treatment (Torres-León et al. 2018, Lebaka et al. 2021). Although anti-nutritional characteristics are reported in mango, this problem can be minimized to maximize the nutritional potential of this ingredient (Felix e Silva et al. 2020, 2022).

Brazil is the seventh-largest producer of mangoes, mangosteens, and guava and the largest producer in America (2.57 million tons) (FAO 2022). In specific periods, the production of mangos can generate surpluses. This high production level generates large quantities of residues or by-products representing up to 50% of the weight of the fruit (Tirado-Kulieva et al. 2022). Besides, many producers need to discard part of their mango production due to the disqualification of the fruit for commercialization. This disposal generates waste dumped into the environment, causing environmental damage (Parfitt et al. 2010). When mangos are considered unsuitable for human consumption, instead of being discarded, they can be used as feed in aquaculture or contribute to the formulation of diets for fish. According to Tirado-Kulieva et al. (2022), even mango by-products are a high nutritional and bioactive value source. Previous studies have already shown their potential of inclusion in fish diets, such as African carp (*Labeo senegalensis*) (Omoregie 2001), Nile tilapia (*Oreochromis niloticus*) (Obasa et al. 2013), pacamã (*Lophiosilurus alexandri*) (Souza et al. 2015), zebrafish (*Danio rerio*) (Lizárraga-Velázquez et al. 2019), and hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) (Khieokhajonkhet 2020).

Aquaculture development in Brazil has the potential to contribute to increasing world demand for fish. Brazil has high freshwater species diversity, and tambaqui (*Colossoma macropomum*) is the most produced native species in continental aquaculture (Buzollo et al. 2018). It occupies the second position among the species for export, with 78 tons of fish exported in 2022, reaching US$ 268,839 (Embrapa 2023). It is an omnivorous freshwater fish from the Amazon Basin.

Some ingredients from fruits have starch residues have already been tested in fish feed for tambaqui in the form of the whole banana (*Musa* sp.) meal (Felix e Silva et al. 2020), crude grape (*Vitis vinifera*) extract (Morante et al. 2021), and crude yellow yam (*Dioscorea cayennensis*) extract (Souza et al. 2023). So, studies are still needed to determine the digestibility of these ingredients and the maximum levels of inclusion, in addition to verifying their effects on the growth and health of fish (Souza et al. 2018, 2021b) to identify the potential of using these sources. Determining the apparent digestibility coefficient (ADC) is the first step in evaluating an ingredient's nutritional quality and utilization efficiency in complete animal diets (Buzollo et al. 2018).

In a previous study, Souza et al. (2018) verified that mango pulp meal replacement for CM in the diet for tambaqui fingerlings (~3.7 g) improved growth performance. However, these authors evaluated only a few metabolic parameters and did not consider the digestibility of the diet. They also assessed the pulp of the mango and not the whole fruit. Our study aimed to evaluate the ADC (crude protein, crude energy, and dry matter) of whole mango meals (WMM) in tambaqui juveniles. We also seek to assess diets containing different proportions of CM substitution by WMM for the growth performance of tambaqui juveniles, which are about ten times heavier compared to tambaqui fingerlings investigated by Souza et al. (2018). In addition, we also analyzed fish health through metabolic, hematological, and intestinal enzyme parameters in these fish.

MATERIALS AND METHODS

Local

The Companhia de Desenvolvimento do Vale do São Francisco e Parnaíba, Petrolina, Brazil supplied the fish. Then, two experiments were conducted at the Laboratório de Aquicultura da Universidade Federal do Vale do São Francisco (UNIVASF) in the same city. The first experiment evaluated WMM digestibility. The second experiment assessed the replacement of CM by WMM. Procedures for handling animals and collecting biological samples were approved by the Ethical Committee of the UNIVASF (protocol n.0016/140415).

During the experiments, the temperature (26.40 ± 1.22° C), dissolved oxygen (5.13 ± 0.20 mg O_2 L^{-1}) (oximeter; Politerm-Pol 60, São Paulo, Brazil), and pH (6.61 ± 0.10) (pH meter; Hanna HI-98130, Barueri, Brazil) were monitored daily. The alkalinity (50.00 ± 0.00 mg $CaCO_3$ L^{-1}) and non-ionized ammonia (0.12 ± 0.04 mg NH_3 L^{-1}) were monitored by kit (Alfatecnoquímica, Florianópolis, Brazil) twice a week. The tanks were cleaned daily by siphon to remove excess feces and feed residues.

Experiment 1 - Digestibility trial

The reference diet (RD) was formulated from standard ingredients to meet the nutritional requirements of tambaqui (Felix e Silva et al. 2020). The experimental diet consisted of 70% RD and 30% WMM (NRC 2011) (Table I). Mangos not used for marketing and human consumption were purchased from a local market. The mangos were rinsed thoroughly in tap water, followed by double distilled water, 10 mL NaClO L^{-1}, and 200 mg Cl L^{-1}. Mango pieces (about 3.0 cm^2) were air dried in an oven at 45° C for 24 h and ground into a fine powder using a grinder. The obtained meal, called WMM (with peel, pulp, and almond), was stored at –20° C until use.

The RD and WMM diets were manufactured using the following sequence: grinding (using a hammer mill of 0.8 mm mesh), weighing and homogenizing (30 min), and adding water (12% of the total weight). The mixture was extruded

Table I. Composition and chemical composition of reference diet (RD) and 30% inclusion of whole mango (*Mangifera indica*) meal (WMM) in the diet.

Nutrient (g kg^{-1})	RD	WMM
Whole soybean meal	326.70	
Poultry viscera flour	198.50	
Corn gluten	100.00	
Corn meal	231.10	
Wheat meal	110.00	
Soybean oil	10.00	
Salt	11.50	
Premix[a]	5.00	
Vitamin C[b]	5.00	
Chromium oxide	1.00	
Antifungal[c]	1.00	
Antioxidant[d]	0.20	
RD (g kg^{-1})	-	700.00
WMM (g kg^{-1})	-	300.00
Variables	Chemical composition	
Crude protein (g kg^{-1})	329.40	290.60
Crude energy (kcal kg^{-1})	41850.00	39930.00
Ethereal extract (g kg^{-1})	30.80	19.70
Dry matter (g kg^{-1})	910.20	916.70
Crude fiber (g kg^{-1})	28.40	30.80

[a] Vitamin and mineral mix (Premix fish Agromix®; Jaboticabal, Brazil) (guaranteed levels per kg of premix) — BHT: 5 mg; nicotinic acid: 20 g; folic acid: 1,200 mg; pantothenic acid: 10 mg; vit A: 2,400 UI; vit D3: 600 UI; vit E: 30 UI mg; vit K3: 3 mg; vit C: 60 g; pantothenic acid: 10 mg; biotin: 200 mg; choline: 100 g; Inositol: 25 g; Vit B1: 4 mg; Vit B2: 4 mg; Vit B12: 8 mg; Vit B2: 4 mg; Vitamin B6: 3 mg; Se: 100 mg; Co: 80 mg; Zn: 24 mg; Fe: 20 g; Cu: 3,500 mg; Mn: 10 mg; I: 160 mg. [b] calcium salt 2-ascorbic acid monophosphate, 420 g kg^{-1} of the active ingredient. [c] Calcium propionate. [d]Butyl-hydroxytoluene.

(90 °C by about 2 s) using a 1.0 mm die plate and then dehydrated in a forced air circulation oven (55° C for 24 h). The pellets (6 mm) were stored under refrigeration (-20 °C). The chemical composition was determined according to AOAC (2016).

Animals and experimental design

The ADC for WMM was determined. The indirect method of digestibility measurement was used, which consists of the total collection of feces using an indigestible marker in the diet (Cr_2O_3). The ADC was estimated by the difference in nutrient marker concentration between food and fish feces (see the calculation in the next section). The difference found in the feces analysis of the fish belonging to the groups RD and WMM was used to estimate the digestibility of WMM.

The RD and WMM diets were conducted in quadruplicate. Eight conical tanks (200 L) were used for the feeding experiments. The fish (45.20 ± 06.00 g; n = 10 per tank) were allowed to adjust to the management conditions and facilities and fed three times daily (8:30, 12:30, and 16:30 h) with a commercial diet (Presence NutrioPiscis, Carpina, PE, Brazil; 28% crude protein) until apparent satiety for two weeks. Then, samples (feces) were collected for ten days to produce pooled samples. The fish were fed under the same conditions as the adjustment period.

The tanks were part of an open flow system, with a total water change every 30 min. Thirty min after the last feeding, the tanks were cleaned, and the water was changed. Feces collectors (Falcon conical tubes of 300 mL) were individually coupled to the bottom of the tanks. They were retrieved and stored within polystyrene coolers containing ice to reduce feces degradation. Sediment was collected in vials overnight and removed the following morning (6:30 h), transferred to plastic bottles, and then stored at - 20 °C. The material was centrifuged at 2,800 *x g* for 10 min. Afterward, feces samples were oven-dried for 48 h, placed in labeled plastic bottles and stored at - 80° C.

Apparent digestibility coefficients (ADC) calculation

The crude protein, crude energy, and dry matter ADC were calculated using chromic-III oxide

(Cr_2O_3) levels in the samples recovered from the feces (NRC 2011). The Cr_2O_3 was determined according to the methodology described by Bremer Neto (2003). After conducting the Cr_2O_3 quantitative analysis and based on the nutrient values present in test diets and feces, the ADCs in the diets were calculated according to the concentration of Cr_2O_3 added to the diets, which was recovered in the feces. The ADC calculations were calculated according to NRC (2011):

$$ADC(n) = 100 - 100 \times (I_D/I_F) \times (N_F/N_D)$$

Where ADC(n) = apparent digestibility coefficient, I_D = concentration of chromium-III oxide in diet (g kg^{-1}), I_F = concentration of chromium-III oxide in feces (g kg^{-1}), N_D = nutrients in the diet, and N_F = nutrients in feces.

$$ADC\ (ing) = [(a + b)\ ADC_{ED} - (a)\ ADC_{RD}]/b$$

Where ADC (ing) = apparent digestibility coefficient of the ingredient, ADC_{ED} = apparent digestibility coefficient of the experimental diet, ADC_{RD} = apparent digestibility coefficient of the RD, a = experimental ingredient percentage, and b = RD percentage.

Experiment 2 - Substitution of corn meal with whole mango meal

Experimental diets were prepared according to Felix e Silva et al. (2020) to meet the minimum nutritional requirement for species at the age studied for crude protein (320-327 g kg^{-1}) and crude energy (4,200-4300 kcal kg^{-1}). A completely randomized design with five treatments in quadruplicate was used. The levels of substitution of CM by WMM in the diets were 0 (control), 80, 160, 240, and 320 g kg^{-1} (Table II). All procedures for milling, extrusion (90°C by 2 s), and drying the diets and chemical composition followed the same methodology as in Experiment 1.

The fish (31.24 ± 0.44 g; n = 10 per tank; n total = 200) were randomly distributed in 20 tanks with a capacity of 1,000 L of water in a water recirculation system, with aeration and mechanical and biological filters for an experimental period of 45 days. The fish were fed three times daily (08:30, 12:30, and 16:30 h) until apparent satiation.

Determination of the concentrations of total flavonoids and phenols

The analysis of total flavonoid and total phenol contents was described in detail by Felix e Silva et al. (2020). The total flavonoid content was expressed as mg quercetin equivalents (QE) g^{-1} through the quercetin calibration curve. The calibration curve was obtained at concentrations ranging from 2.5 to 20 µg mL^{-1} (R^2 = 0.993). The gallic acid calibration curve expressed the total phenol content as mg gallic acid equivalents (GAE) g^{-1}. The calibration curve was obtained at 50 to 1000 mg L^{-1} (R^2 = 0.997).

Growth performance

At the end of the experimental period (Day 45), the fish fasted for 24 h before the growth performance analysis and collection of blood, carcass, intestine, and liver samples. The weight (g) of tambaqui from all experimental units was measured to calculate growth performance. The growth performance variables were calculated using the following formulae:

Weight gain (WG, g) = Final body weight (g) – Initial body weight (g);

Specific growth rate (SGR, % per day–1) = 100 × (Ln final weight (g) – Ln initial weight (g)) / Time (days);

Carcass yield (CY, %) = 100 × [(Carcass weight (g) / Fish weight (g)]);

Survival (%) = 100 × (Final fish number/ Initial fish number).

Table II. Composition of experimental diets containing different levels of whole mango (*Mangifera indica*) meal (WMM).

Ingredient (g kg^{-1})	WMM (g kg^{-1})				
	0	80	160	240	320
Whole soybean meal	200.00	200.00	200.00	200.00	200.00
Poultry by-product meal	200.00	200.00	200.00	200.00	200.00
Corn meal	323.10	254.80	186.50	118.20	50.00
Wheat meal	100.70	100.70	100.70	100.70	100.70
Albumin	105.20	105.20	105.20	105.20	105.20
WMM	0.00	80.00	160.00	240.00	320.00
Soybean oil	14.00	12.30	10.50	8.80	7.00
Premix[a]	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	5.00	5.00	5.00	5.00	5.00
Choline chloride	2.00	2.00	2.00	2.00	2.00
DL-Methionine	1.80	1.80	1.90	1.90	1.90
Vitamin C[b]	5.00	5.00	5.00	5.00	5.00
Antifungal[c]	1.00	1.00	1.00	1.00	1.00
Cellulose	40.00	30.00	20.00	10.00	0.00
Antioxidant[d]	0.20	0.20	0.20	0.20	0.20
Chemical composition (g kg^{-1})					
Crude protein (g kg^{-1})	326.80	325.20	323.40	321.70	320.00
Crude energy (kcal kg^{-1})	4,199	4,213	4,246	4,279	4,298
Ethereal extract (g kg^{-1})	55.50	55.10	54.70	54.40	53.90
Crude fiber (g kg^{-1})	67.10	67.90	68.70	69.50	70.20
Mineral matter (g kg^{-1})	47.00	46.20	45.30	44.40	43.50
Dry matter (g kg^{-1})	909.40	900.60	901.80	903.0	904.10
Total phenols (mg GAE g^{-1})[e,*]	8.75	9.97	12.10	14.73	14.58
Total flavonoids (mg QE g^{-1})[f,*]	2.33	2.89	3.71	13.35	17.08

[a]Vitamin and mineral mix (Premix fish Agromix®; Jaboticabal, Brazil) (guaranteed levels per kg of premix) — folic acid: 1200 mg; nicotinic acid: 20 g; pantothenic acid: 10 mg; BHT: 5 mg; vit A: 2400 UI; vit D3: 600 UI; vit E: 30 UI mg; vit K3: 3 mg; vit C: 60 g; pantothenic acid:10 mg; biotin: 200 mg; choline: 100 g; Inositol: 25 g; Vit B1: 4 mg; Vit B2: 4 mg; Vit B12: 8 mg; Vit B2: 4 mg; Vit B6: 3 mg; Co: 80 mg; Fe: 20 g; Se: 100 mg; Cu: 3500 mg; Mn: 10 mg; Zn: 24 mg; I: 160 mg. [b]calcium salt 2-ascorbic acid monophosphate, 420 g kg^{-1} of the active ingredient. [c]Calcium propionate. [d]Butyl-hydroxytoluene. [e]GAE = gallic acid equivalent. [f]QE = quercetin equivalents. * = Linear regression. Equations: Total phenols: y = 8.802 + (0.0203x), R^2 = 0.94, p = 0.007. Total flavonoids: y = – 0.120 + (0.0499x), R^2 = 0.85, p = 0.026.

Sample collection

Three fish from each tank (n = 12 per treatment) were randomly sampled. The juveniles were removed from the tank and anesthetized with benzocaine hydrochloride (30 mg L^{-1}). The blood (1.5 mL) was collected from the caudal vessel using heparinized syringes (5000 UI). The samples were divided into two aliquots.

One blood aliquot (0.50 mL) was used for hematological analyses, and the second aliquot (1.00 mL) was used to obtain plasma for the biochemical determinations. Both aliquots were transferred to 2.50 mL polyethylene tubes. The second aliquot of blood was centrifuged at 4°C at 3000 *x* g for 10 min to separate the plasma and stored under refrigeration at –80°C. After blood collection, the fish were euthanized by a lethal benzocaine hydrochloride (250 mg L^{-1}) dose and spinal cord sectioning. The liver and whole intestinal tract samples were collected and preserved at –80°C until analysis.

Hematological and plasmatic analysis

Erythrocytes (Ery) were counted using a Neubauer chamber. Hematocrit (Hct) was determined using the method of microhematocrit with centrifugation at 12,000 *x* g for 5 min in a microhematocrit centrifuge (Micro spin, Model Spin 1000, Jaboticabal, Brazil). The hemoglobin (Hb) concentration was determined using the cyanmethemoglobin method and spectrophotometer readings at 540 nm absorbance. These data were used for determining the hematimetric indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) according to the following formulae: mean corpuscular volume (MCV, fL) = Hct × 10 / Ery (×10^6 µL), mean corpuscular hemoglobin (MCH, pg) = Hb × 10 / Ery and mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}) = Hb × 100 / Hct.

Determinations of plasmatic glucose, triglycerides, total proteins, albumin, and cholesterol were performed using commercial kits (Labtest® kits; Vista Alegre, Brazil), and all absorbance readings were performed on a semi-automatic biochemical analyzer (Doles®, Model D-250, Goiânia, Brazil). The procedure for plasma determinations was carried out as follows: plasma glucose by the glucose oxidase method and absorbance reading at 520 nm; total protein using the colorimetric method of biuret reaction and absorbance reading at 545 nm; albumin, triglycerides, and cholesterol using the colorimetric method with absorbance reading at 630, 540, and 500 nm, respectively. Plasmatic amino acid concentrations were determined according to Copley (1941) using a spectrophotometer at 570 nm.

Hepatic and intestinal analysis

The intestine was collected entirely, with a cut of approximately 10 cm from the stomach, and after a cross-sectional cut, to obtain a sample of approximately 100 mg. The fish were fasting, so the intestine was not washed. Liver and intestine (whole tract) samples (100 mg) were homogenized in a buffer (10 mmol L^{-1} phosphate/20 mmol L^{-1} Tris, pH 7.0) using a mechanical homogenizer (Marconi MA039, Piracicaba, Brazil) before centrifugation at 600 *x* g for 3 min at 4°C. The supernatant was centrifuged at 6000 *x* g for 10 min for hepatic aspartate aminotransferase (AST) and intestinal enzyme analysis.

The enzymatic activity of AST was measured in the buffered extracts using commercial kits (Labtest®). The reading was performed at 340 nm in a Bel Photonics® semi-automatic biochemical analyzer (Model 2000 UV, Monza, Italy). Hepatic glycogen was determined according to Bidinotto et al. (1997), with spectrophotometer readings at 480 nm absorbance.

The intestinal amylase activity was determined spectrophotometrically at 660 nm using a commercial kit (Amylase Bioclin®; BioclinQuibasa, Belo Horizonte, Brazil). The intestinal nonspecific alkaline protease was determined using kits (Alkaline protease, Labtest®). The enzymatic activity was defined as the amount of enzyme needed to catalyze the formation of 1 mg min^{-1}.

Statistical analysis

The results are expressed as the mean ± standard error of the mean (SEM). Levene's test verified the homoscedasticity of the variances. The data showing homogeneous variances were compared using one-way analysis of variance (ANOVA), followed by post hoc Tukey tests ($p < 0.05$). As the treatments are quantitative independent variables with graded levels of WMM in the diet, significant results ($p < 0.05$) were compared using orthogonal polynomial contrasts. The best model was based on the

p-value and R^2 values. All values were used to determine the linear or quadratic effects of the different treatments tested.

RESULTS

Digestibility

No mortality occurred. The crude energy and dry matter ADC of WMM was significantly higher than the RD ($p < 0.05$). Crude protein did not present significant differences between treatments. In addition, the crude protein, crude energy, and dry matter ADC were above 91% and 96% for RD and WMM, respectively (Fig. 1).

Inclusion of WMM

No mortality occurred. There was no effect from the inclusion of WMM in tambaqui diets on growth performance (final weight, weight gain, and DGR) and carcass yield ($p > 0.05$) (Table III). The inclusion of WMM provided a positive linear effect on plasma glucose, amino acids, and albumin values and a negative linear effect on plasma cholesterol levels, hepatic glycogen, and AST activity ($p < 0.05$). A quadratic effect was verified for plasma triglyceride levels, where the lowest values were found for fish that received between 80 and 240 g WMM kg diet^{-1} ($p < 0.05$) (Table IV).

Fish that received 320 g WMM kg diet^{-1} showed plasma glucose and amino acid levels significantly higher than those of the control group or those fed with 80 and 160 g WMM kg diet^{-1} ($p < 0.05$). In addition, plasma amino acid levels were significantly lower in the control group than in other groups ($p < 0.05$). Hepatic glycogen levels were significantly higher in the control group than in the treatment with 240 g WMM kg diet^{-1} ($p < 0.05$). Plasma total protein levels and intestinal amylase and alkaline protease enzyme activity were not influenced by the inclusion of WMM in diets (Table IV).

The inclusion of WMM resulted in a negative linear effect for hemoglobin and MCHC ($p < 0.05$) (Table V). A quadratic effect was verified for erythrocytes, where there was an increase in the values of the treatments 80 and 160 g WMM kg diet^{-1} ($p < 0.05$). Hematocrit, MCV, and MCH were not influenced by the different treatments ($p > 0.05$) (Table V).

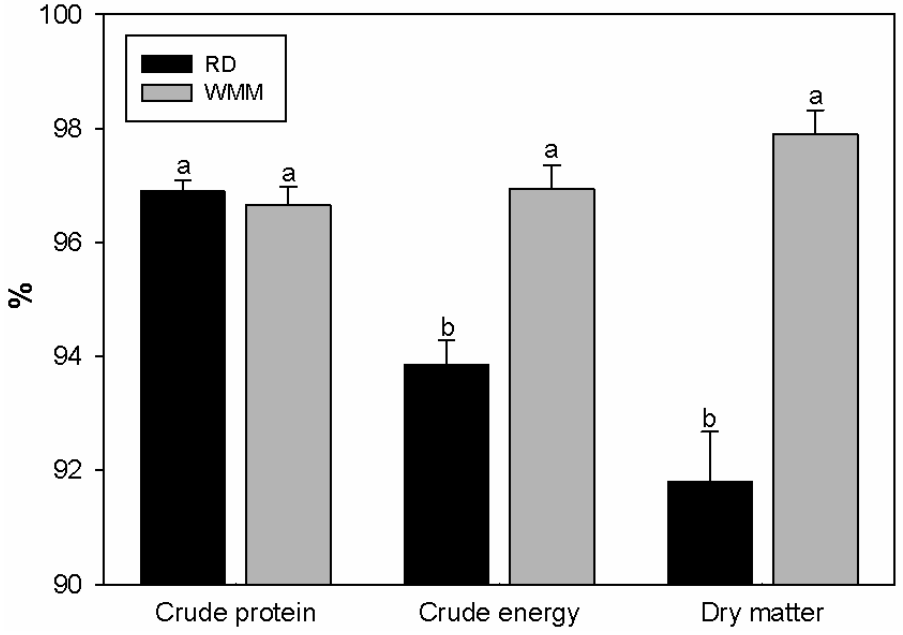

Figure 1. Apparent digestibility coefficient (mean ± SEM) in the reference diet (RD) and whole mango (*Mangifera indica*) meal (WMM; 30% inclusion) in tambaqui (*Colossoma macropomum*) juveniles. Different letters indicate statistical differences between treatments (Tukey's test, $p < 0.05$; n = 4 tanks per treatment).

Table III. Growth performance (mean ± SEM) of juvenile tambaqui (*Colossoma macropomum*) submitted to different levels of inclusion of whole mango (*Mangifera indica*) meal (WMM) for 45 days.

Variables	WMM (g kg^{-1})				
	0	80	160	240	320
IW	31.20±0.51	30.95±1.43	31.60±0.66	31.20±1.25	31.25±0.50
FW	58.04±1.03	57.07±1.96	59.16±1.47	58.97±1.73	56.59±1.52
WG	26.84±0.52	26.12±0.85	27.56±0.81	27.77±0.50	25.34±1.02
SGR	1.38±0.02	1.36±0.03	1.39±0.02	1.42±0.02	1.32±0.02
CY	90.45±0.33	90.75±0.39	91.71±0.38	91.46±0.36	91.60±0.32

IW (initial weight), FW (final weight), and WG (weight gain) are expressed in g. SGR (specific growth rate) is expressed as % per day. CY (carcass yield) is expressed in %. n = 4 tanks per treatment.

Table IV. Biochemical blood plasma, hepatic, and digestible enzymes (mean ± SEM) of tambaqui (*Colossoma macropomum*) fed with diets containing different levels of the whole mango (*Mangifera indica*) meal (WMM) for 45 days.

Variables	WMM (g kg^{-1})				
	0	80	160	240	320
Plasma					
Glucose*	90.23±2.70^{b}	88.39±4.61^{b}	90.23±3.33^{b}	103.37±6.40^{ab}	110.83±3.03^{a}
Total proteins	3.84±0.15	3.88±0.21	4.16±0.20	4.22±0.25	4.01±0.16
Amino acids*	17.20±0.79^{c}	19.83±0.67^{b}	20.67±0.56^{b}	21.85±0.38^{ab}	24.00±0.56^{a}
Albumin*	1.32±0.09	1.30±0.08	1.35±0.04	1.36±0.04	1.40±0.06
Triglycerides**	376.33±19.42	337.99±25.38	330.30±13.32	343.00±20.36	393.49±24.69
Cholesterol*	144.10±9.57	142.04±6.07	127.71±3.21	124.77±5.02	124.65±4.11
Liver					
Glycogen*	9.83±0.57^{a}	7.78±0.55^{ab}	7.64±0.65^{ab}	6.35±0.66^{b}	7.64±0.81^{ab}
AST activity*	1.74±0.24	1.59±0.21	1.59±0.33	1.34±0.30	1.29±0.18
Intestine					
Amylase	5.58±0.53	5.60±0.70	5.65±0.38	6.45±0.47	5.47±0.62
Alk protease	4.73±0.37	4.69±0.30	4.67±0.49	4.68±0.58	4.68±0.45

Glucose and triglycerides are expressed as mg dL^{-1}. Total proteins and albumin are expressed as g dL^{-1}. Amino acids are expressed as nmol mL^{-1}. Cholesterol is expressed as mg dL^{-1}. Glycogen is expressed as µmol glucose g tissue^{-1}. AST (aspartate aminotransferase) is expressed as U L^{-1}. Amylase and alk (alkaline) protease enzymes are expressed in UI mg protein^{-1}. Different letters indicate a statistical difference between treatments (Tukey's test, p < 0.05). n = 12 fish per treatment. * = Linear regression. ** = Quadratic regression. Equations: Glucose: y = 85.368 + (0.703x), R^2 = 0.80, p = 0.041. Amino acids: y = 17.643 + (0.195x), R^2 = 0.96, p = 0.003. Albumin: y = 1.302 + (0.00275x), R^2 = 0.82, p = 0.035. Triglycerides: y = 376.648 – (6.582x) + (0.221x^2), R^2 = 0.99, p < 0.01. Cholesterol: y = 143.888 – (0.702x), R^2 = 0.85, p = 0.025. Glycogen: y = 9.186 – (0.0948x), R^2 = 0.79, p = 0.043. AST activity: y = 1.741 – (0.0145x), R^2 = 0.92, p = 0.010.

DISCUSSION

Digestibility

This is the first study that evaluated WMM digestibility in tambaqui. Both tested diets presented high ADC values (> 91%) for crude protein, crude energy, and dry matter, which was expected because tambaqui can efficiently digest several types of feed (Guimarães et al. 2014). We found that WMM reflected good nutrient utilization with indices above 96% digestibility.

Our findings for crude protein and crude energy ADC were above the values reported for CM in tambaqui by Buzollo et al. (2018), 94.5 and 88.7%, respectively. In addition, crude protein ADC decreased when fermented mango seed meal was used to substitute CM in practical diets of Nile tilapia, while ADC carbohydrate was increased (Obasa et al. 2013). Therefore, in our study, crude protein, crude energy, and dry matter ADC indicated good digestibility.

Dry matter ADC measures the total quantity of an ingredient that is digested and absorbed and a high dry matter ADC may indicate a lower fiber concentration (Chen et al. 2016). Few studies have estimated dry matter digestibility in tambaqui diets despite their relative importance. In this sense, for tambaqui, Guimarães et al. (2014) found values of dry matter digestibility ranging from 42.4% to 83.6% in eight different feeds, including CM (77.8%) and Felix e Silva et al. (2020) found 91.8% and 94.3% in CM and whole banana meal, respectively.

The increased crude energy ADC for the WMM could be due to the high amounts of a simple carbohydrate source such as the fructose of this fruit (Souza et al. 2018). Starch is critical CM's most important non-fibrous polysaccharide (Hertrampf and Piedad-Pascual 2000). Based on the crude energy ADC of the present study, we verified that fructose might have been digested more efficiently than starch. It is due to the exchange of sources that results in different carbohydrate monomers whose transport mechanisms, metabolism, and absorption are different for each molecule (Souza et al. 2021a). In previous studies, the crude energy ADC values for CM in tambaqui were lower (76.4% and 88.70%) (Guimarães et al. 2014, Buzollo et al. 2018) than the value observed in the present study. These divergent results occur because the digestibility of food may vary due to its composition, feeding conditions, or fish species (Souza et al. 2021b).

The presence of phenolic compounds (9.97-14.73 mg GAE g^{-1}) and flavonoids (2.89-17.08 mg QE g^{-1}) of WMM in our study did not impair its ADC for tambaqui. Fibers and anti-nutritional factors (e.g., phytates, tannins, and keratins) are relevant to animal nutrition and can influence ADC values (Lima et al. 2011). It is a fact that mangos have anti-nutritional factors like tannin and phytate (Khieokhajonkhet 2020). These compounds reduce the nutritional value of foods by interfering with the digestibility of proteins, carbohydrates, and minerals they bind (Torres-León et al. 2018). However, based on the high ADC values found for tambaqui in

Table V. Hematological variables (mean ± SEM) of tambaqui (*Colossoma macropomum*) fed with diets containing different levels of the whole mango (*Mangifera indica*) meal (WMM) for 45 days.

Variables	WMM (g kg^{-1})				
	0	80	160	240	320
Hct	28.58±0.62	29.25±0.55	29.42±0.36	29.33±0.41	28.08±0.42
Ery**	1.91±0.10	1.96±0.10	1.98±0.08	1.84±0.05	1.91±0.10
Hb*	13.80±0.64	13.12±0.28	12.96±0.63	12.95±0.68	12.14±0.69
MCV	155.41±10.77	153.16±8.48	151.54±6.72	160.27±4.38	152.48±9.90
MCH	73.95±4.11	68.75±4.07	67.28±4.98	71.39±5.05	65.38±4.87
MCHC*	48.34±2.05	44.93±0.96	44.19±2.31	44.38±2.72	43.24±2.44

Hct (Hematocrit) is expressed as %. Ery (erythrocytes) concentration is expressed as 10^6 μL^{-1}. Hb (hemoglobin) concentration and MCHC (mean corpuscular hemoglobin concentration) are expressed as g dL^{-1}. MCV (mean corpuscular volume) is expressed in fL. MCH (mean corpuscular hemoglobin) is expressed in pg. n = 12 fish per treatment. * = Linear regression. ** = Quadratic regression. Hct: $y = 28.533 + (0.134x) - (0.00456x^2)$, $R^2 = 0.95$, $p = 0.047$. Hb: $y = 13.694 - (0.0438x)$, $R^2 = 0.87$, $p = 0.020$. MCHC: $y = 47.165 - (0.134x)$, $R^2 = 0.77$, $p = 0.049$.

this study, the digestibility of WMM and CM was unaffected by crude fiber or polysaccharide content. Furthermore, tambaqui can efficiently digest fiber (Felix e Silva et al. 2020).

In the current study, the extrusion process (90 °C in this study) may have inactivated anti-nutritional factors. In addition, extrusion can trigger serial events such as starch gelatinization, inactivation of anti-nutritional factors, expanded non-structural carbohydrates, and formation of amylose-lipid complexes (Francis et al. 2001, da Silva et al. 2021). Gelatinization is the combination of changes (rupture of the granular structure of starch molecules, their expansion, hydration, and solubilization) that can disrupt the cell wall of plant cells and increase the susceptibility of the nutrients to digestion by digestible enzymes and the stability of the feed pellets in water (Zongjia & Hardy 2003). Therefore, extruded diets (such as those evaluated in this study) may increase nutrient digestibility, mainly in carbohydrates (da Silva et al. 2021). In addition, tambaqui is an omnivorous species with a tendency toward being an herbivore (Souza et al. 2021a), which favored the digestibility of the diets as seen in our results, which demonstrated high values of crude protein and crude energy ADC in WMM. While the energetic components in practical diets for tambaqui have considerable importance (Nascimento et al. 2020), WMM can contribute to meeting fish's dietary requirements.

WMM inclusion

Since the volume of mango by-products is a problem, their correct management is essential to generating economic profits and reducing environmental pollution. In addition, the use of alternative foods influences fish growth performance. However, fish diets can be affected by inclusion levels, anti-nutritional factors, fiber, and secondary compounds of plant metabolism (Felix e Silva et al. 2020, Souza et al. 2023). In the current study, although digestibility was improved, fish growth did not differ between treatments. This could have been influenced by the duration of the experiment (45 days). Therefore, we suggest that future experiments increase their duration by at least 60 days. Our results showed a progressive increase in these compounds with the inclusion of WMM in the diet. The presence of anti-nutritional factors (flavonoids and phenols) in diets with higher levels of WMM (240 and 320 g kg diet^{-1}) may have harmed the fish development in these treatments because these factors commonly affect palatability, feed intake, nutrient retention, and metabolism (Kokou & Fountoulaki 2018). On the other hand, mango contains polyphenolic compounds with high antioxidant activity (Lizárraga-Velázquez et al. 2019, Tirado-Kulieva et al. 2022), which may have counterbalanced the anti-nutritional factors because the replacement of CM by WMM at any of the levels evaluated maintained the same growth performance of the juveniles investigated in this study.

Our study illustrated that WMM is a food that can be used in practical diets for tambaqui juveniles. Unlike our results, the inclusion of mango pulp in the diet provided higher performance in tambaqui fingerlings fed with 300-400 g kg^{-1} (Souza et al. 2018) and in Nile tilapia up to 150 g kg^{-1} (Lima et al. 2011). Similarly, replacing CM with mango seed meal did not affect red hybrid tilapia and Nile tilapia growth rates (Khieokhajonkhet 2020, Eyiwunmi et al. 2021). On the other hand, a negative effect on Nile tilapia performance with the inclusion of values above 330 g WMM kg^{-1} was reported (Souza et al. 2013).

The WMM used in the present study contained peel, pulp, and almond. The mango peel has high tannin levels (Rashmi et al. 2017); therefore, it could decrease the activity

of digestive enzymes and cause changes in the mucosa of the digestive tract (Mandal & Ghosh 2010). However, this has not been verified in this study because our diets were extruded to reduce the deleterious effects of tannins on fish digestion (Francis et al. 2001), avoiding inhibition of enzymatic activity. In our study, therefore, this anti-nutritional factor was generally insufficient to inhibit intestinal amylase and alkaline protease activity.

In the current study, fish fed with 240 or 320 g WMM kg diet^{-1} had glucose values slightly higher than the reference values (until 102.9 mg L^{-1}) described by Tavares-Dias (2015). Sugars such as fructose and sucrose present in fruits are commonly converted to glucose. Tambaqui reduces its glycemia slowly, and the time it takes for blood glucose to be regulated is moderately long, with the fish remaining hyperglycemic longer because it is intolerant to a high concentration of fructose (Souza et al. 2021a, 2023). Therefore, we believed that fish fed diets with the highest levels of CM replacement by WMM might be mildly hyperglycemic. In addition, plasma triglyceride levels were increased in the 320 g WMM kg diet^{-1} treatment. In fish, prolonged hyperglycemia may result from a diet with high carbohydrate levels (Walker et al. 2020). It should be avoided as it reduces food intake and causes physiological alterations related to changes in brain glucosensing markers (Polakof et al. 2011). In addition, high carbohydrate levels can raise plasma triglyceride levels (Souza et al. 2021, 2023), which agrees with our findings in the treatment with the highest inclusion of WMM.

Despite the increase in plasma glucose and triglycerides levels, other metabolic parameters showed an inverse behavior in the present study. Plasma cholesterol and liver glycogen levels are affected by the same factors as glucose and triglycerides (Walker et al. 2020) and were reduced as the percentage of replacement of CM by WMM in the diet of juveniles increased. Hepatic glycogen reserves can regulate plasma glucose maintenance. The reduced hepatic glycogen levels in fish fed diets with high concentrations of WMM could result from decreased oxygen availability in plasma (Walker et al. 2020), which enhances glycogen utilization by increasing carbohydrate consumption. This finding is consistent with some of our hematological values (erythrocytes, hemoglobin, and MCHC values) that were reduced when fish were fed with 240 or 320 g WMM kg diet^{-1}. Despite this, the present study's hematological parameters were in the reference range for the species (Tavares-Dias 2015).

Regarding the metabolic parameters evaluated in the current study, as there was no change in the exogenous source of plasma total protein levels, the concentrations of this metabolite remained unchanged from their concentrations in plasma. Still, plasma amino acid and albumin levels increased when WMM was added to the diet. Tambaqui can effectively use amino acids of proteic or energetic ingredients (Nascimento et al. 2020), which was reflected in our study using diets with WMM inclusion. Plasma amino acid leads to better use of the carbohydrate source for the energetic process and the use of available amino acids for fish growth (Souza et al. 2018). The plasma total protein concentration is related to protein metabolism and nutritional conditions (Morante et al. 2021), and albumin is the main nutrient-carrying protein that directly reflects the animal's nutritional status (Yilmaz & Ergün 2012). Reducing plasma albumin levels could harm tambaqui metabolism since this protein is a vital antioxidant in blood and extracellular fluids (Halliwell 1988).

In the present study, although plasma albumin levels were reduced, other results that should be regarded as positive when CM was

replaced with WMM were the reduction of the hepatic activity of the AST enzyme and plasma cholesterol levels. The transamination process catalyzed by AST is linked to protein turnover and deamination metabolism. This process is essential to obtain carbon skeletons aimed at gluconeogenesis and oxidative processes (Melo et al. 2016, Falco et al. 2020), where its release into the bloodstream can increase the response of a possibly harmful effect on liver tissue (Chung et al. 2021). A reduction in plasma cholesterol levels can be explained by the presence of soluble fibers found in mango, which increase bile acids to remove cholesterol from the plasma (Souza et al. 2018). This reduction is associated with fish's nutritional status and can prevent liver pathologies related to fat accumulation in the liver in fish fed with an artificial diet (Zhai et al. 2016, Chung et al. 2021). The presence of WMM in practical diets for tambaqui contributes to liver health.

CONCLUSIONS

The WMM digestibility of dry matter, crude protein, and crude energy was considered high (> 96%). Replacement CM by WMM did not result in differences in fish growth rate. Therefore, the WMM may be a viable alternative as an energy ingredient for tambaqui juveniles' diet, especially when its use is economically advantageous, either using surplus fruits or fruits that would not be used for human consumption. In an integrative view of metabolic and hematological results, we recommended that WMM could replace up to 16% of CM without causing hyperglycemia or physiological changes in tambaqui juveniles.

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RSM and CEC: statistical analysis, supervised the findings, discussion, and final text. EPO.: conducted the experiments and hematobiochemical analysis. DRR, ASR, AMS, and ACSC: collaboration on data sampling, analysis conduction, results, and discussion. JFBM: conception and design and supervised the findings. All the authors have read and approved the manuscript.