HSOA Journal of Aquaculture & Fisheries

Research Article

Different Concentrations of Protein and Fresh Mango as a Carbohydrate Source in the Tambaqui Diet (*Colossoma Macropomum*)

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Abstract

A 45 – day feed study aimed to evaluate the effects of cornmeal and in *natura* mango at different concentrations of carbohydrates and proteins on performance, hematology, metabolism and digestive enzymes of tambqui, *Colossoma macropomum*. In the experiment, 144 juveniles were randomly distributed in a factorial scheme, where eight experimental diets (C30P30, C36P28, C42P26, C48P24, M30P30, M36P28, M42P26 and M48P24) were tested for 45 days.

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Performance parameters, hematological indices, metabolites and digestive enzyme activity were evaluated. The best performance was observed in the fish that received the diet C42P26, with cornmeal as a carbohydrate source. The results of glucose, triglycerides and total plasma protein had a significant effect on levels, source and interaction effect. The levels of triglycerides and cholesterol were higher in the diets with fresh mango. The activity of the enzyme glutamate dehydrogenase presented variations, modulated from the carbohydrate and protein concentrations of the diet. The digestive enzymes activity adapted to the profile of the diets tested and were not related to fish performance. It is concluded that cornmeal diet (C42P26) is the best source of carbohydrate, promoting better performance and health of C. *macropomum*..

Keywords: Alternative feeding; Digestive enzymes; Hematology; Metabolism

Introduction

Fish feed represents between 50-70% of the total production cost [1], due to the use of high levels of protein ingredients (fishmeal) and the preparation process used [2]. The addition of carbohydrate sources and levels has been studied, principally for omnivorous fish, since adequate inclusion levels can improve the efficiency on the use of other nutrients that make up the feed. Fish present a lower efficiency in metabolizing carbohydrates than mammals; however, the beneficial effects can be gained from carbohydrate supplementation in the diets [3].

Dietary carbohydrate utilization may vary according to the dietary habits of the species, as well as the source and the level of carbohydrates in the diet [4]. Fish diets supplemented with carbohydrates help reduce protein and lipid catabolism, provide metabolites for biological synthesis and reduces the costs of feed production [5-8]. Cornmeal is rich in carbohydrates and widely used as a raw material in several industrial products [9], among them is commercial fish feed, as an energy source [10].

There are other alternative carbohydrate sources, such as fruits, that have been studied in fish feed. They are collected from those deemed unfit for human consumption and have already been tested in *Oreochromis niloticus* [11], *Colossoma macropomum* [12, 13] and *Leporinus obtusidens* [14]. Among the fruits used, mango (*Mangifera indica*) stands out and it is commercialized *in natura* or in a processed form. In its composition are equivalent carbohydrate values of 16.5 g for every 100 g [15]. In the Haden and Tommy cultivars, sugars such as glucose, fructose and sucrose are found in proportions of 0.59%, 3.15% and 9.05%, respectively, and protein values of 4.4g kg^{-1} in pulp [16]. Thus, the nutritional value of fruit residues can be a good alternative for use in feed, and it is necessary to investigate the use of these ingredients in performance and digestibility assays for better utilization of these residues.

Regarding the species under study, tambaqui (*Colossoma macropomum*) is a native fish from the Amazon and Orinoco rivers, of omnivorous feeding habits, with herbivore and frugivore tendencies [17]. This species presents excellent growth performance and

resistance to hypoxia [18, 19], favorable characteristics for the cultivation of this species in different breeding systems. Therefore, the aim of this study was to evaluate the effect of varying carbohydrate sources and levels plus different protein concentrations over performance, hematological, metabolic responses and activities of digestive enzymes of juvenile tambaqui.

Materials and Methods

For this experiment, 144 tambaqui juveniles with an initial average weight of 3.93 ± 0.5 g were used. The fish were randomly distributed in 24 PVC tanks with a capacity of 500 L in a closed system with recirculating, biofiltered water. Each experimental unit was composed of six juvenile tambaqui. Eight experimental diets (C30P30, C36P28, C42P26, C48P24, M30P30, M36P28, M42P26 and M48P24) were formulated varying two carbohydrates sources (corn meal - C and *in natura* mango - M) in four different levels (30, 36, 42 e 48%) and four crude protein (P) concentrations of 30, 28, 26 and 24%. The diets was formulated (Table 1) to meet the nutritional requeriments of *Colossoma macropomum*, according to [20].

The mango used to make the rations was unfit for human consumption (production rejects). After acquiring the mango, they were crushed and mixed with the other ingredients. It was carried out by taking advantage of the fruit humidity in the preparation of the experimental diets. The values of the *in natura* mango composition were determined on the basis of the dry matter for inclusion in the feed. After mixing, the ingredients were pelleted in a ball-type grinder, then dried in a forced-air recirculation oven for 24 hours at 55 ^{0}C, and finally crushed to fit the pellet for the fish's mouth.

The tambaqui juveniles were fed twice a day (9:00 a.m. and 4:00 p.m.) up to apparent satiation for 45 days. 30 minutes after feeding, the boxes were siphoned daily for the removal of feces and possible feed leftovers. The water quality parameters were monitored during the experimental period using a multiparameter probe. All the experimental procedures performed with the fish were authorized by the Ethics Committee for Animal Use (CEUA) of the Federal University of São Francisco Valley, protocol number 0016/140415.

At the end of the experimental period the tambaqui juveniles were weighed to evaluate the growth performance through the following parameters: Total weight gain (TWG), Average daily weight gain (ADWG), Specific growth rate (SGR) and Condition factor (CF).

For the analysis of metabolic parameters and metabolic digestive enzymes we withdrew from six juvenile tambaqui, from each treatment, biological tissues (intestine and liver) plus blood for obtaining plasma.

The fish were subjected to blood collection puncture of the caudal vein with heparinized syringes. The plasma was obtained by blood centrifugation at 5000 rpm for 5 minutes and then was frozen at -20 ^{0}C for later analysis. Soon the fish were anesthetized with benzocaine (1g 10L^{-1}) and euthanized for liver and intestine collection.

For the assessment of hematological parameters, we used the blood collected to determine red blood cell (RBC) count, hematocrite and hemoglobin. From this data we calculated hematimetric parameters, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) according to the formulas describes by [21]. The hematocrit (Ht) was performed as described by [22], the heparinized

Dietary level protein (%)	30	28	26	24	30	28	26	24
Dietary level CHO (%)	30	36	42	48	30	36	42	48
Ingredient (%)	Corn meal				In nature mango			
Fish meal	11.41	15.21	22.85	30.64	10.63	14.23	19.59	26.87
Soybean meal	47.99	38.19	23.37	8.24	47.29	37.40	25.23	10.13
Corn meal	30.00	36.00	42.00	48.00	_	_	_	_
In natura mango	_	_	_	_	30.00	36.00	42.00	48.00
Soybean oil	7.13	7.59	8.77	10.03	8.43	9.14	10.18	11.78
L-lysine	_	_	_	0.05	_	_	_	0.15
DL-methionine	_	_	_	0.03	_	_	_	0.06
Common salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix-APP 1	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin C 2	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Bicalcium phosphate	0.46	_	_	_	0.64	0.22	_	_
BHT 3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Nutrients	Calculated nutritional composition							
Dry matter (%)	94.91	94.22	94.95	93.97	88.27	85.17	92.58	92.20
Gross energy (Kcal kg-1)	4.200	4.200	4.200	4.200	4.200	4.200	4.200	4.200
Crude protein (%)	30.00	28.00	26.00	24.00	30.00	28.00	26.00	24.00
Crude fiber (%)	2.92	2.37	1.54	0.70	2.87	2.31	1.63	0.79
Total carbohy-drates(%)	44.48	45.15	43.69	42.10	46.09	47.10	47.14	45.94
Lysine (%)	1.95	1.80	1.65	1.52	1.90	1.73	1.57	1.52
Methionine (%)	1.33	1.14	0.89	0.68	1.30	1.11	0.89	0.68

Table 1: Formulation and calculated composition of experimental diets on the based dry matter.

1Premix min. and vit. (mineral and vitamin mix): Guarantee levels per kilogram of product: Vit. A, 1,200,000 IU; Vit. D3, 200,000 IU; Vit. E, 12,000mg; Vit. K3, 2400mg; Vit. B1, 4,800mg; Vit. B2, 4,800mg; Vit. B6, 4,000mg; Vit. B12, 4,800mg; B.C. Folic acid, 1,200mg; Pantothenate Ca, 12,000mg; Vit. C, 48,000mg; Biotin, 48mg; Hill, 65,000mg; Niacin, 24,000mg; Iron, 10,000mg; Copper, 6,000mg; Manganese, 4,000mg; Zinc, 6,000mg; Iodine, 20mg; Cobalt, 2mg; Selenium, 20mg; 2Vitamin C protected: calcium salt 2-monophosphate of ascorbic acid, 42% active principle (calcic salt, ascorbicacid2-monophosphate-42% activeprinciple); 3Butyl-Hydroxytoluene;

micro-capillary tube is filled 2/3 of its total volume and soon after the sealing was centrifuged at 12000 rpm for 5 minutes. Hemoglobin concentration (Hb) by the cyanomethabhamoglobin method described by [23] using the Drabkin reagent and reading was performed at 540nm. The red blood cell counts were made in a Neubauer chamber, using the [24] reagent.

Plasma measured metabolites were glucose, albumin, triglycerides, protein and total cholesterol following colorimetric methods of the reagents (labtest®). The free amino acids were determined following the [25] methodology, where a 1mm glycine standard was used and the extract was added to a 0.1% solution of ninhydrin in propanol and the reading was performed on a spectrophotometer at 570nm.

The hepatic glycogen (HG) determinations were performed as described by [26]. The liver samples from each fish were weighed in the

proportion of 100 mg and then transferred to test tubes to dissolution. 100µl of this extract was transferred to a tube and added with 250µl of ethanol and 100 µl of 10% K_2SO_4, followed by stirring. Then, the sample was centrifuged at 3000 rpm for 3 minutes. Subsequently, the supernatant was discarded by inversion and the precipitate resuspended in 2 ml of distilled water. After mixing 100 µl of the sample, 250 µl of phenol were transferred plus 1 ml of H_2SO_4 to stop the reaction, finally the spectrophotometer was read at 480 nm.

To determine the activity of the enzyme glutamate dehydrogenase (GDH), liver samples weighing approximately 100 mg were homogenized in buffer (10 mM phosphate/20 mM Tris-pH 7.0) at 4 C using a mechanical homogenizer. The activity of the GDH enzyme was determined according to [27]. The reaction is based on the reduction of 2-ketoglutarate to glutamate. The reaction medium contained imidazole-HCl buffer pH 7.7-50 mM, 250 mM ammonium acetate, 0.1 mM NADH, 1 mM ADP, 0.5 mM NADP 0.5 mM 2-ketoglutarate. To determine the activity, 100 microliters of the homogenate was used, and the reading was performed at a wavelength of 360nm.

For determination of digestive enzymes intestine samples weighing approximately 100 mg were homogenized in 10 mM phosphate buffer / 20 mM tris-pH 7.0 at 4 C using mechanical homogenizer. The intestinal amylase activity was determined according to the method proposed by [28]. With 1.0 ml of starch solution in 0.1M tris buffer (pH 7.0) containing 0.02M NaCl , a suitable volume of cell homogenate was added and the reaction mixture was incubated for 40 minutes at 25 C. After the reaction time, 250 µl of 15% trichloroacetic acid (TCA) was added and the reaction mixture was centrifuged at 3000 g for 2 minutes. In the supernatant, the glucose concentration was estimated by the [29] method.

To determine the intestinal alkaline proteolytic activity, a 1% casein solution was used as the reaction substrate. The incubation mixture was composed of 250 – 400 µL of 1% azocasein, 0.1 Tris/HCl buffer (pH 8.0). After incubation of the mixture for 30 minutes at 35 C, the reaction was stopped by adding 1.0 ml of 15% TCA, then centrifuged at 1800 g for 10 minutes [30]. Tyrosine was used as the standard and the unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1µg of tyrosine per minute.

Non-specific intestinal lipase activity was determined according to the method described by [31]. The reaction was incubated at 35 ^{0}C in medium containing 0.4 mM p-nitrophenyl meristat in a buffer solution of 24 mM ammonium bicarbonate, pH 7.8 and 0.5% Triton X-100. After 30 minutes, the reactions were stopped by the addition of 25 mM NaOH. The spectrophotometer reading was performed at 405nm.

The design used in this experiment was entirely random in a factorial scheme (4x2), with four protein levels (30, 36, 42 and 48%) and two carbohydrates sources (cornmeal or mango *in natura*) with three replicates. The results were analyzed by the computer program *Statistical Analysis System* - SAS (Version 9.1, 2003). The normality of residues was previously verified by the SHAPIRO-WILK test (PROC UNIVARIATE) and the variances were compared by orthogonal contrasts (linear, quadratic and deviation of the quadratic and source effect) with a significance level of 5% by PROC GLM. When significant, an interaction effect level versus source was deployed. Subsequently the contrast analyses, when significant, determined the parameters of the regression equations by PROC REG.

Results

The results obtained in the analysis of water quality parameters during the experiment were: electrical conductivity 91.2 ± 2.1 mS, total salinity 43.61 ± 1.8 ppm, dissolved oxygen 6.3 ± 0.6 mg L^{-1}, ammonia concentration 0.059 ± 0.007 mg L^{-1}, nitrite 0.044 ± 0.008 mg L^{-1}, phosphorus 0.286 ± 0.33 mg L^{-1}, nitrate 2.832 ± 0.238 mg L^{-1}, pH 7.23 ± 0.3 and average temperature of 26.1 ± 4.78 ^{0}C.

There was a significant difference and interaction effect (P<0.05) in all parameters of zootechnical performance evaluated (Table 2). Fish presented better performance (TWG, ADWG and CF) in the diet C42P26%. With the *in natura* mango diet, the best result was in the diet M36P28.

The carbohydrate sources and the protein levels tested influenced the hematological parameters of tambaqui juveniles (Table 3). Mean hematocrit values were significant at levels and sources. The hemoglobin level and the number of erythrocytes showed influence (P<0.05) in levels, sources and showed interaction. The results of mean corpuscular volume (MCV) showed interaction (P <0.05) in levels. Mean values of mean corpuscular hemoglobin (MCH) concentrations were only significant (P <0.05) between sources.

The results of metabolic intermediates plasma, liver glycogen (HG) and enzyme activity glutamate dehydrogenase (GDH) activity measured in liver are shown in Table 4. The results of total analyzed glucose, triglycerides and total plasma proteins were significantly affected by source, levels and interaction effect. The glucose levels are higher in the inclusion of cornmeal treatments. The concentrations of triglycerides were higher in treatments (M42P26 and M48P24) with inclusion of fresh mango.

Plasma cholesterol had an effect (P<0.05) in relation to the sources, where the highest concentrations were in the fish fed with fresh mango. Plasma albumin concentrations were significant (P<0.05) in sources and levels, which decreased with increasing carbohydrate levels and reduction of dietary protein in both carbohydrate sources tested. The plasma proteins presented a quadratic effect the two tested carbohydrate sources. Total amino acid levels showed significant interaction Table 5.

The results of HG and GDH presented difference (P<0.05) in levels and sources showed interaction. The HG levels are increased in fish receiving higher amounts of the cornmeal diet. However, the animals that were fed the highest levels of fresh mango and lower concentration of protein, presented less deposition in the liver.

The GDH enzyme activity was higher in fish fed with diets containing cornmeal. The greatest enzymatic activities occurred at the lowest and highest concentrations of carbohydrates and protein.

The activities of digestive enzymes presented significant differences (P <0.05). The alkaline protease and the amylase were influenced in levels, sources and presented interaction effect. Lipase was significantly affected on levels and interaction.

Discussion

Some food and their concentrations influence the fish's performance, due to their bromatological characteristics. In addition, the nutrient contents present are responsible for the productive responses. In the present study, the zootechnical parameters evaluated were higher in fish that received the cornmeal and lower in the fish fed fresh

Variables	C30P30	C36P28	C42P26	C48P24	MSE	M30P30	M36P28	M42P26	M48P24	MSE
TWG (g)	29.63	33.49	36.31	29.14	0.75	19.12	23.67	6.52	7.93	0.98
ADWG (g)	0.63	0.71	0.77	0.62	0.02	0.41	0.50	0.14	0.17	0.02
SGR (%)	4.54	4.78	4.85	4.87	0.05	3.73	4.01	1.95	1.91	0.13
CF	1.90	1.54	1.46	1.53	0.03	1.46	2.02	1.46	1.79	0.04
Effect (Probability)										
Rates	Linear	Quadratic		Levels	Sources	L * S	RE			R2
TWG	*	*		*	*	*	C= -81.846+5.999x -0.076x2 M= 21.217+0.526x-0.017x2			0.44 0.51
ADWG	*	*		*	*	*	C= -1.741+0.127-0.001x2 M= 0.441+0.011-0.000x2			0.31 0.51
SGR	*	*		*	*	*	C= 4.099+0.016x M= 7.775-0.125x			0.23 0.61
CF	NS	NS		NS	*	*	C= 6.854-0.256x+0.003x2 M= -4.697 + 0.327x -0.004x2			0.61 0.33

Table 2: Growth performance of tambaqui juveniles fed diets containing different sources and levels of carbohydrates and reduction of crude protein levels.

TWG: Total Weight Gain; ADWG: Daily Average Gain Gain; SGR: Specific Growth Rate; CF: Condition Factor. MSE: Medium Standard Error; *: P <0.05; NS: Not Significant.

Variables	C30P30	C36P28	C42P26	C48P24	MSE	M30P30	M36P28	M42P26	M48P24	MSE
Hct1	25.00	25.75	23.20	20.75	0.49	22.40	22.00	13.80	16.75	0.93
Hb2	17.22	16.55	20.20	14.75	0.56	13.98	12.51	8.00	10.92	0.49
RBC3	2.21	4.38	3.88	3.65	0.18	3.03	2.53	2.29	2.00	0.12
MCV4	99.07	52.77	76.72	56.18	4.90	73.55	73.05	57.58	86.11	3.05
MCH5	94.63	43.23	53.80	40.73	4.80	43.41	57.45	35.59	51.94	2.27
MCHC6	68.40	96.38	76.37	64.32	3.81	62.28	57.48	55.16	69.81	2.31
Effect (Probability)										
Rates	Linear	Quadratic	Levels	Sources	L * S	RE			R2	
Hct	*	NS	*	*	NS	C= 33.593-0.253x M= 35.790-0.439x			0.55 0.51	
Hb	NS	*	*	*	*	C= -22.146+2.141x-0.028x2 M= 62.90-2.477x+0.028x2			0.38 0.62	
RBC	NS	*	*	*	*	C= -23555600.000+1363733.33x-16666.67x2 M= 6802566.667-170322.222x+1472.222x2			0.66 0.42	
MCV	*	*	*	NS	*	C= 417.408-16.680x + 0.194x2 M= 353.736-15.251x + 0200x2			0.38 0.33	
MCH	NS	*	*	NS	NS	Y= 549.211-23.280x+0.266x2			0.71	
MCHC	NS	NS	NS	*	*	C= -312.928+21.142x-0.277x2 M= 247.384-10.198x+0.135x2			0.34 0.23	

Table 3: Hematological parameters of tambaqui juveniles fed diets containing different sources and levels of carbohydrates and reduction of crude protein levels.

1Hct: Hematocrit (%); 2Hb: Hemoglobin (g/dL); 3RBC: Red blood cells (106/µL); 4MCV: Medium Corpuscular Volume (fL); 5MCH: Medium Corpuscular Hemoglobin (pg); 6MCHC(g/dL-1): Mean Corpuscular Hemoglobin Concentration.

MSE: Medium Standard Error; CP: Crude Protein; *: p<0.05; NS: Not Significant.

Citation: Silva AFE, Melo JFB, Souza AMD, Melo FVST, Pereira GA, et al. (2022) Different Concentrations of Protein and Fresh Mango as a Carbohydrate Source in the Tambaqui Diet (Colossoma Macropomum), J Aquac Fisheries 6: 045.

• Page 5 of 8 •

Variables	C30P30	C36P28	C42P26	C48P24	MSE	M30P30	M36P28	M42P26	M48P24	MSE
GLI1	106.96	97.12	110.18	114.15	2.98	112.31	99.40	84.46	85.11	2.85
HG2	261.12	264.45	265.32	274.81	1.79	269.83	264.42	263.63	259.27	1.96
TRI3	135.43	110.24	142.49	171.06	6.53	153.31	156.45	241.57	284.98	13.65
COL4	63.21	67.04	76.81	90.14	3.55	121.64	105.70	114.11	109.06	3.88
ALB5	0.93	0.85	0.80	0.63	0.03	0.84	0.65	0.65	0.48	0.03
PROT6	3.43	3.54	3.88	3.21	0.13	3.13	3.60	3.00	2.84	0.09
AAT7	34.64	39.54	32.00	28.56	1.50	28.99	29.93	32.78	41.08	1.38
GDH8	378.09	388.35	178.48	420.48	23.33	200.27	94.75	127.08	230.36	12.23
Effect (Probability)										
Rates	Linear	Quadratic	Levels	Sources	L * S	RE				R2
Glucose	*	*	*	*	*	C=307.100+9.502x + 0.101x2 M=154.164-1.503x				0.86 0.76
Hepatic Glycogen	NS	NS	NS	*	*	C=239.171+0.698x M=285.390-0.541x				0.30 0.21
Triglycerides	*	*	*	*	*	C=49.360+2.319x M=-103.004+8.002x				0.25 0.67
Cholesterol	NS	NS	NS	*	NS	C=74.30 M=112.62				– –
Albumin	*	NS	*	*	NS	C=1.410-0.015x M=1.354-0.017x				0.37 0.64
Proteins	*	*	*	*	*	C=-4.732+0.443x-0.006x2 M=-2.534+0.326x+0.005x2				0.33 0.35
Total Amino acids	NS	NS	NS	NS	*	C=-35.192+4.095x-0.058x2 M=83.187-3.333x+0.051x2				0.24 0.51
GDH Activity	NS	*	*	*	*	C=2770.427-126.903x+1.609x2 M=2223.623-111.056x+1.450x2				0.27 0.85

Table 4: Results of plasma metabolic intermediates, hepatic glycogen and GDH enzyme activity.

1Glucose (mg.dL-1), 2Hepatic Glycogen (mg/g/tec), 3Triglycerides (mg.dL-1), 4Cholesterol (mg.dL-1), 5Albumin (g.dL-1), 6Total Proteins (g.dL-1), 7Total Amino Acids (µl.mL), 8Glutamate Dehydrogenase (U.g-1 prot). MSE: Medium Standard Error; *: P <0.05; NS: Not Significant.

Enzymes	C30P30	C36P28	C42P26	C48P24	MSE	M30P30	M36P28	M42P26	M48P24	MSE
Alkaline Protease	84.15	75.22	68.22	44.57	4.56	62.44	57.70	26.18	44.47	4.18
Amylase	0.32	0.37	0.42	1.80	0.16	0.47	0.38	1.26	0.92	0.09
Lipase	2.88	2.41	2.02	1.03	0.19	1.86	1.67	1.43	3.01	0.17
Effect (Probability)										
	Linear	Quadratic	Levels	Sources	L * S	RE			R2	
Alkaline protease	*	*	*	*	*	C= -1.051+ 5.874x-0.102x2 M= 339.197 - 13.894x + 0.159x2			0.68 0.48	
Amylase	*	*	*	*	*	C= 11.347-0.640x + 0.009x2 M= -3.170 + 0.001x – 0.168x2			0.93 0.48	
Lipase	*	*	*	NS	*	C= 0.609 + 0.182x - 0.003x2 M= 18.081-0.907x + 0.012x2			0.82 0.78	

Table 5: Activity of intestinal enzymes (IU/mg protein) in tambaqui juveniles fed diets containing different sources and levels of carbohydrates and reduced on crude protein levels.

EPM: Medium Standard Error; *: P <0.05; NS: Not Significant.

mango, in the extent of C42P26 and M36P28, respectively. This dietary influence over zootechnical indexes has already been reported in Nile tilapia (*Oreochromis niloticus*) substituting cornmeal with fresh mango peel flour [32], in Hungarian carp (*Cyprinus carpio*) with tung meal [33], in Nile tilapia (*Oreochromis niloticus*) substituting cornmeal for mango residue meal [34].

The effects of carbohydrate sources and their relation to dietary protein can be positive or not in fish growth, because they depend on their inclusion levels. In addition, they may be related to the feeding habit of the species and the nutritional composition of the ingredient. It is difficult to define the sources and levels of inclusion of carbohydrates in fish feed due to some factors such as physical state, composition and molecular complexity of the carbohydrate sources, which directly influence digestibility, absorption and metabolism of nutrients [4, 14].

Regarding fish hematological and hematometric variables, they can reflect to the nutritional states caused by food, nutrients and other physiological and pathological conditions. In this study, the sources tested and the protein levels used modified the profile of the red series. From the adjusted equations, we can observe the adjustment of the red series to the dietary conditions. The adaptations of hematological and hematimetric variables already were been described in tambaqui juveniles fed with diets containing leucine leaf meal [35] in Nile tilapia fed with diets containing triticale levels instead of corn [36].

The change in RBC variables, hemoglobin level, and hematocrit was higher in cornmeal fed animals, which may be related to higher performance. Erythrocytes contained the hemoglobin that carries oxygen (O_2) [37] to the tissues and cells, which provides greater metabolization of the molecules. The hematological status is important for assessing fish's nutritional and health status [38], physiological state [39] and environmental conditions [40]. It is probable that in the tambaquis fed with *in natura* mango, the red series was impaired and compensatory adaptations were achieved, such as reduction of RBC, but with an increase of mean corpuscular volume and hemoglobin concentration. All this adaptation may have occurred due to the lower utilization of this food.

Another important aspect in fish nutrition studies is the metabolic response, which reflect and respond to the different types of food and nutrients present in a diet. The adaptation of metabolic parameters in fish caused by feeding has already been described by several authors [41, 42, 14]. The increase in glycemia was observed in juveniles of *Megalobrama amblycephala* fed with different carbohydrate sources [3]. Plasma glucose levels are directly related to osmoregulatory variations, presence of stress factors and diet composition [43]. In this study, a reduction in hepatic glycogen reserve was observed in fish fed with fresh mango. The reduction of hepatic glycogen reserve is related to type of carbohydrate in the diet and the ability of the species to mobilize glycogen for the maintenance of glucose homeostasis [44, 45].

Plasma triglycerides in the two sources tested higher for higher carbohydrate-lower protein concentrations. According to [43] excess glucose is converted to lipids through lipogenesis, and this induction may occur after feeding and directly involves glucose homeostasis [46]. Plasma total cholesterol showed only interaction between the sources and not between the diet carbohydrate and protein concentrations. Cholesterol is changed by the types of lipids present in the diet [47, 46]. The observed changes are within reference levels in fish [33, 12].

Other important metabolites are albumin and plasma protein, as they are directly related to fish nutritional status. Albumin responded with a linear decline in the two tested sources and protein levels in the diet. According to [48] albumin is a high-density lipoprotein, and its synthesis is influenced by nutrition, hormonal balance, general liver status, and stress. Total protein presented a quadratic effect. Its reference values are related to metabolic strategies of transport and tissue damage, and especially in malnutrition [37, 49]. The total plasma protein is altered mainly by changes in plasma volume [50]. It is likely that the reduction in these metabolites in this study happen at greater concentration of the two carbohydrate sources, and the that lower levels of dietary protein promoted smaller amounts of nutrients provided in the digestion.

The change in the plasma free amino acid profile presented different responses in the two foods tested. In the highest concentrations of cornmeal there was a reduction of the plasma amino acids. However, fish that received higher concentrations of fresh mango had increased amino acids in the plasma. The reduction of this metabolic with the use of the mango denotes mobilization for use in the energy processes, since the performance of these animals was lower, suggesting gluconeogenesis. This fact has been described by [51] in rainbow trout and Nile tilapia fed rations with different protein proportions. Amino acids can be used for energy synthesis, in addition to other compounds [19, 52].

Another response that denoted the use of amino acids as an energy source by tissues is the GDH enzymatic activity. GDH activity occurred in both the highest and the lowest carbohydrate and protein concentrations for both sources tested. The GDH activity is indicative of amino acid deamination for energy purposes. The reduction of GDH enzyme activity in rainbow trout and Nile tilapia fed diets with lower protein levels [51] and the increase of enzyme activity in *Dicentrarchus labrax* with high carbohydrate protein ratio [53]. Thus, it appears that the sources tested and the protein concentrations in this study produce a profile of energy utilization from increased GDH activity.

Some authors have described changes in the activity of digestive enzymes due to the composition of the diet used to feed fish [54-56]. In this study the reduction in nonspecific alkaline protease activity may be related to the reduction of protein levels in the rations. The substitution of foods of protein origin in diets for *Argyrosomus regius* presented a similar response in proteases, reducing activities [56]. The activity of amylase and lipase digestive enzymes in this study did not present a clear relationship with the tested diets, they were only responsive. In a study with *Odontesthes bonarienses* the amylase activity is reduced when it decreases the carbohydrate levels in the diet [57-59]. [7] found a reduction in lipase enzyme activity in *Larmichthys crocea* when fed diets containing lower lipid levels. The result of the present study reinforces that the diet composition directly influences fish digestive enzyme activity. The enzymatic responses of the tambaqui digestive system presented seem to adapt to the foods and nutrients tested, however, they do not present a clear relation with the performance.

Conclusion

In summary the diet C42P26 is better for tambaqui performance. The concentrations of *in natura* mango in the diet modify the erythrocytes, hemoglobin and hematocrit, however without damaging the health of this specie. The analyzed metabolites were responsive to

the tested diets as a performance strategy. The reduction of amino acid deamination occurred in the diets C42P26 and M36P28 where the fish reached its highest performance in the sources tested. The activity of digestive enzymes modifies their profile without presenting clear relation to food and protein concentration, relative to performance.

Acknowledgements

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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