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In Natura Mango, Cornmeal and Protein Levels in Tambaqui Diet

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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. 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 performanceand health of *C. macropomum*.

INTRODUCTION

Fish feed represents between 50-70% of the total production cost[1], due to the use of high levels of protein ingredients (fish-meal) 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 (*Mangífera indica*) stands out andit 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], 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 tambaquis.

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 500L in a closed system with recirculating, biofiltered water. Each experimental unit was composed of six juvenile tambaquis. Eight experimental diets (C30P30, C36P28, C42P26, C48P24, M30P30, M36P28, M42P26 and M48P24) were formulate dvarying 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 Guimaraes and Martins[19].

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 h at 55°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 min 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 multi-parameter 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

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

	Values							
Experimental diets	1	2	3	4	5	6	7	8
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 carbohydrates(%)	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

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

5 min and then was frozen at -20°C for later analysis. Soon the fish were anesthetized with benzocaine (1g10 L^{-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 Wintrobe[20]. The Hematocrit (Ht) was performed as described by Goldenfarb *et al*.[21], the heparinized micro-capillary tube is filled 2/3 of its total volume and soon after the sealing was centrifuged at 12000 rpm for 5 min. Hemoglobin concentration (Hb) by the cyanomethabhamoglobin method described by Drabkin[22] using the Drabkin reagent and reading was performed at 540 nm. The red blood cell counts were made in a Neubauer chamber, using the Natt and Herrick[23] reagent.

Plasma measured metabolites were glucose, albumin, triglycerides, proteinand total cholesterol following colorimetric methods of the reagents (labtest®). The free aminoacids were determined following the Copley[24] methodology where a 1 mm 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 570 nm. The Hepatic Glycogen (HG) determinations were performed as described by Bidinotto *et al*.[25]. The liver samples from each fish were weighed in the proportion of 100 mg and then transferred to test tubes to dissolution. The 100 µL of this extract was transferred to a tube and added with 250 µL of ethanol and 100 µL of 10% K_2SO_4, followed by stirring. Then, the sample was centrifuged at 3000 rpm for 3 min. Subsequently, the supernatant was discarded by inversion and the precipitate re-suspended 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 Hochachka *et al*.[26]. 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 mL of the homogenate was used and the reading was

performed at a wavelength of 360 nm. 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 Hidalgo *et al*.[27]. With 1.0 mL of starch solution in 0.1 M tris buffer (pH 7.0) containing 0.02 M NaCl , a suitable volume of cell homogenate was added and the reaction mixture was incubated for 40 min 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 min. In the supernatant, the glucose concentration was estimated by the Park and Johnson[28] 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 min at 35°C, the reaction was stopped by adding 1.0 mL of 15% TCA, then centrifuged at 1800 g for 10 min[29]. 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 1 µg of tyrosine per minute.

Non-specific intestinal lipase activity was determined according to the method described by Gawlicka *et al*.[30]. The reaction was incubated at 35°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 min, the reactions were stopped by the addition of 25 mM NaOH. The spectrophotometer reading was performed at 405 nm.

The design used in this experiment was entirely random in a factorial scheme (4×2) 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°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.

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 (Table 5).

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

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	485	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	R^2
TWG	*	*	*	*	*	C = -81.846+5.999x-0.076x^2	0.440.51
						M = 21.217+0.526x-0.017x^2	
ADWG	*	*	*	*	*	C = -1.741+0.127-0.001x^2	0.310.51
						M = 0.441+0.011-0.000x^2	
SGR	*	*	*	*	*	C = 4.099+0.016x	0.230.61
						M = 7.775-0.125x	
CF	NS	NS	NS	*	*	C = 6.854-0.256x+0.003x^2	0.610.33
						M = -4.697+0.327x -0.004x^2	

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

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

Variables	C30P30	C36P28	C42P26	C48P24	MSE	M30P30	M36P28	M42P26	M48P24	MSE
Hct[1]	25.00	25.75	23.20	20.75	0.49	22.40	22.00	13.80	16.75	0.93
Hb[2]	17.22	16.55	20.20	14.75	0.56	13.98	12.51	8.00	10.92	0.49
RBC[3]	2.21	4.38	3.88	3.65	0.18	3.03	2.53	2.29	2.00	0.12
MCV[4]	99.07	52.77	76.72	56.18	4.90	73.55	73.05	57.58	86.11	3.05
MCH[5]	94.63	43.23	53.80	40.73	4.80	43.41	57.45	35.59	51.94	2.27
MCHC[6]	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	R^2
Hct	*	NS	*	*	NS	C = 33.593-0.253x	0.55
						M = 35.790-0.439x	0.51
Hb	NS	*	*	*	*	C = -22.146+2.141x-0.028x^2	0.38
						M = 62.90-2.477x+0.028x^2	0.62
RBC	NS	*	*	*	*	C= -23555600.000+1363733.33x-16666.67x^2	0.66
						M = 6802566.667-170322.222x+1472.222x^2	0.42
MCV	*	*	*	NS	*	C = 417.408-16.680x+0.194x^2	0.38
						M = 353.736-15.251x+0200x^2	0.33
MCH	NS	*	*	NS	NS	Y = 549.211-23.280x+0.266x^2	0.71
MCHC	NS	NS	NS	*	*	C = -312.928+21.142x-0.277x^2	0.34
						M = 247.384-10.198x+0.135x^2	0.23

[1]Hct: Hematocrit (%); [2]Hb: Hemoglobin (g dL^{-1}); [3]RBC: Red blood cells (10^6 µ L^{-1}); [4]MCV: Medium Corpuscular Volume (fL); [5]MCH: Medium Corpuscular Hemoglobin (pg); [6]MCHC(g dL^{-1}): Mean Corpuscular Hemoglobin Concentration; MSE: Medium Standard Error; CP: Crude Protein; *: p<0.05; NS: Not Significant

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

Variables	C30P30	C36P28	C42P26	C48P24	MSE	M30P30	M36P28	M42P26	M48P24	MSE
GLI[1]	106.96	97.12	110.18	114.15	2.98	112.31	99.40	84.46	85.11	2.85
HG[2]	261.12	264.45	265.32	274.81	1.79	269.83	264.42	263.63	259.27	1.96
TRI[3]	135.43	110.24	142.49	171.06	6.53	153.31	156.45	241.57	284.98	13.65
COL[4]	63.21	67.04	76.81	90.14	3.55	121.64	105.70	114.11	109.06	3.88
ALB[5]	0.93	0.85	0.80	0.63	0.03	0.84	0.65	0.65	0.48	0.03
PROT[6]	3.43	3.54	3.88	3.21	0.13	3.13	3.60	3.00	2.84	0.09
AAT[7]	34.64	39.54	32.00	28.56	1.50	28.99	29.93	32.78	41.08	1.38
GDH[8]	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	R^2
Glucose	*	*	*	*	*	C = 307.100+9.502x+0.101x^2	0.86
						M = 154.164-1.503x	0.76
Hepatic Glycogen	NS	NS	NS	*	*	C=239.171+0.698x	0.30
						M = 285.390-0.541x	0.21
Triglycerides	*	*	*	*	*	C = 49.360+2.319x	0.25
						M = -103.004+8.002x	0.67

Table 4: Continue

Effect (Probability)							
Rates	Linear	Quadratic	Levels	Sources	L*S	RE	R^2
Cholesterol	NS	NS	NS	*	NS	C = 74.30	-
						M = 112.62	-
Albumin	*	NS	*	*	NS	C = 1.410-0.015x	0.37
						M = 1.354-0.017x	0.64
Proteins	*	*	*	*	*	C = -4.732+0.443x-0.006x^2	0.33
						M = -2.534+0.326x+0.005x^2	0.35
Total amino-acids	NS	NS	NS	NS	*	C = -35.192+4.095x-0.058x^2	0.24
						M = 83.187-3.333x+0.051x^2	0.51
GDH Activity	NS	*	*	*	*	C = 2770.427-126.903x+1.609x^2	0.27
						M = 2223.623-111.056x+1.450x^2	0.85

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

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

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)							
Paremeters	Linear	Quadratic	Levels	Sources	L * S	RE	R^2
Alkaline protease	*	*	*	*	*	C = -1.051+5.874x-0.102x^2	0.68
						M = 339.197-13.894x+0.159x^2	0.48
Amylase	*	*	*	*	*	C= 11.347-0.640x+0.009x^2	0.93
						M = -3.170+0.001x-0.168x^2	0.48
Lipase	*	*	*	NS	*	C = 0.609+0.182x-0.003x^2	0.82
						M = 18.081-0.907x+0.012x^2	0.78

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

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 zoo technical parameters evaluated were higher in fish that received the cornmeal and lower in the fish fed fresh mango, in the extent of C42P26 and M36P28, respectively. This dietary influence over zoo technical indexes has already been reported in *Nile tilapia* (*Oreochromis niloticus*) substituting cornmeal with fresh mango peel flour[31], in Hungarian carp (Cyprinus carpio) with tung meal[32], in *Nile tilapia* (*Oreochromis niloticus*) substituting cornmeal for mango residue meal[33].

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 werebeen described in tambaqui juveniles fed with diets containing leucine leaf meal[34] in *Nile tilapia* fed with diets containing triticale levels instead of corn[35].

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)[36] 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[37], physiological state[38] and environmental conditions[39]. 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[44, 41, 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[42]. 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[43, 44].

Plasma triglycerides in the two sources tested higher for higher carbohydrate-lower protein concentrations. According to Polakof *et al*.[42] excess glucose is converted to lipids through lipogenesis and this induction may occur after feeding and directly involves glucose homeostasis[45]. 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[46, 45]. The observed changes are within reference levels in fish[32, 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 Hasegawa *et al*.[47] 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[36, 48]. The total plasma protein is altered mainly by changes in plasma volume[49]. 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 Figueiredo-Silva *et al*.[50] in rainbow trout and Nile tilapia fed rations with different protein proportions. Aminoacids can be used for energy synthesis, in addition to other compounds[51, 52].

Another response that denoted the use of aminoacids 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[50] 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, 55]. In this study, the reduction in non-specific 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]. Zhou *et al*.[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.

REFERENCES

01. De Almeida, L.C., I.M. Avilez, C.A. Honorato, T.S.F. Hori and G. Moraes, 2011. Growth and metabolic responses of tambaqui (*Colossoma macropomum*) fed different levels of protein and lipid. Aquacult. Nutr., 17: e253-e262.

02. Xavier, T.O., M. Michelato, L.V. Vidal, V.R. Furuya and W.M. Furuya, 2014. Apparent protein and energy digestibility and amino acid availability of commercial meat and bone meal for Nile tilapia, *Oreochromis niloticus*. J. World Aquacult. Soc., 45: 439-446.
03. Ren, M., H.M. Habte-Tsion, J. Xie, B. Liu and Q. Zhou *et al*., 2015. Effects of dietary carbohydrate source on growth performance, diet digestibility and liver glucose enzyme activity in blunt snout bream, *Megalobrama amblycephala*. Aquaculture, 438: 75-81.
04. NRC, 2011. Nutrient Requirements of Fish. National Academy Press, Washington DC.
05. Krogdahl, A., G.I. Hemre and T.P. Mommsen, 2005. Carbohydrates in fish nutrition: Digestion and absorption in postlarval stages. Aquacult. Sci., 11: 103-122.
06. Tian, L.X., Y.J. Liu, H.J. Yang, G.Y. Liang and J. Niu, 2012. [Effects of different dietary wheat starch levels on growth, feed efficiency and digestibility in grass carp (*Ctenopharyngodon idella*) (In Portuguese)]. Aquacult. Int., 20: 283-293.
07. Zhou, P., M. Wang, F. Xie, D.F. Deng and Q. Zhou, 2016. Effects of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of large yellow croaker (*Larmichthys crocea*). Aquaculture, 452: 45-51.
08. Li, X., X. Zhu, D. Han, Y. Yang, J. Jin and S. Xie, 2016. Carbohydrate utilization by herbivorous and omnivorous freshwater fish species: A comparative study on gibel carp (*Carassius auratus* gibelio. var CAS III) and grass carp (*Ctenopharyngodon idellus*). Aquacult. Res., 47: 128-139.
09. Butolo, J.E., 2010. [Quality of Ingredients in Animal Feed]. Mundo Agro Editora, Sao Paulo, Brazil, Pages: 430 (In Portuguese).
10. Hardy, R.W. and F.T. Barrows, 2002. Diet Formulation and Manufacture. In: Fish Nutrition, Halver, J.E. and R.W. Hardy (Eds.). 3rd Edn., Academic Press, London, pp: 505-600.
11. Santos, E.L., M.D.C.M. Ludke, J.M. Barbosa, C.B.V. Rabello and J.V. Ludke, 2009. [Apparent digestibility of coconut bran and guava residue by Nile tilapia (*Oreochromis niloticus*) (In Portuguese)]. Rev. Caatinga, 22: 175-180.
12. Bezerra, S.K., R.C. Souza, J.F.B. Melo and D.F.B. Campeche, 2014. [Growth of tambaqui fed different levels of mango flour and protein in the feed (In Portuguese)]. Arch. Zootecnia, 63: 587-598.
13. Souza, S.A., R.C. Souza, D.F.B. Campeche, R.M.L. Campos and J.F.B. Melo, 2014. [Protein: Carbohydrate ratio in the performance and metabolism of hybrids of *Pseudoplatystoma fasciatum* (female) X *Leiarius marmoratus* (male) (In Portuguese)]. Brazil. Arch. Vet. Med. Anim. Sci., 66: 879-886.
14. Lazzari, R., J. Uczay, R.B. Rodrigues, D. Pianesso, T.D. Adorian and P.I. Mombach, 2015. [Use of fruit residue in diets for Piava (In Portuguese)]. Boletimdo Inst. Pesca, 41: 227-237.
15. Marques, A., G. Chicaybam, M.T. Araujo, L.R.T. Manhaes and A.U. Sabaa-Srur, 2010. [Proximate and mineral composition of peel and mango pulp (*Mangifera indica* L.) cv. Tommy Atkins (In Portuguese)]. Rev. Brasileira Fruticultura,` 32: 1206-1210.
16. Bernardes-Silva, A.P.F., F.M. Lajolo and B.R. Cordenunsi, 2003. [Evolution of the levels of starch and soluble sugars during the development and ripening of different mango cultivars (In Portuguese)]. Food Sci. Technol., 23: 116-120.
17. Nunes, E.D.S.S., B.A.S. Cavero, M. Pereira-Filho and R. Roubach, 2006. Exogenous digestive enzymes in the feeding of tambaqui juveniles. Braz. Agric. Res., 41: 139-143.
18. Araujo-Lima, C. and M. Goulding, 1998. [The Fruits of Tambaqui: Ecology, Conservation and Cultivation in the Amazon]. Sociedade Civil de Mamiraua, Brasilia, Pages: 186 (In Portuguese).
19. Guimaraes, I.G. and G.P. Martins, 2015. Nutritional requirement of two Amazonian aquacultured fish species, *Colossoma macropomum* (Cuvier, 1816) and *Piaractus brachypomus* (Cuvier, 1818): A mini review. J. Applied Ichthyol., 31: 57-66.
20. Wintrobe, M.M., 1934. Variations on the size and hemoglobin content of erythrocytes in the blood various vertebrates. Folia Hematol., 51: 32-49.
21. Goldenfarb, P.B., F.P. Bowyer, E. Hall and E. Brosious, 1971. Reproducibility in the hematology laboratory: The microhematrocrit determination. Am. J. Clin. Pathol., 56: 35-39.
22. Drabkin, D.L., 1948. The standardization of hemoglobin measurement. Am. J. Med. Sci., Vol. 215, No. 1. 10.1097/00000441-194801000-00017
23. Natt, P.M. and C.A. Herrik, 1952. A new blood diluent for counting the erythrocytes and leucocytes of chicken. Poult. Sci., 31: 735-738.
24. Copley, N.G., 1941. Alloxan and ninhydrin test. Analyst, 66: 492-493.
25. Bidinotto, P.M., R.H.H. Souza and G. Moraes, 1997. Hepatic glicogen in eight tropical freshwater teleost fish: A procedure for field determinations of microsamples. Boletim Tecnico CEPTA, 10: 53-60.
26. Hochachka, P.W., M. Guppy, H.E. Guderley, K.B. Storey and W.C. Hulbert, 1978. Metabolic biochemistry of water-vs. air-breathing fishes: Muscle enzymes and ultrastructure. Can. J. Zool., 56: 736-750.
27. Hidalgo, M.C., E. Urea and A. Sanz, 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquacult., 170: 267-283.

28. Park, J.T., and M.J. Johnson, 1949. Submicro determination of glucose. J. Biol. Chem., 181: 149-151.
29. Walter, H.E., 1984. Proteinases: Methods with Hemoglobin, Casein and Azocoll as Substrates. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.)., Verlag Chemie, Weinheim, Germany, pp: 270-277.
30. Gawlicka, A., B. Parent, M.H. Horn, N. Ross, I. Opstad and O.J. Torrissenc, 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): Indication of readiness for first feeding. Aquaculture, 184: 303-314.
31. Souza, R.C., J.F.B. Melo, N.R.M. Filho, D.F.B. Campeche and R.A.C.R. Figueiredo, 2013. [Influence of mango flour on the growth and body composition of Nile tilapia (In Portuguese)]. Arch. Zootecnia, 62: 217-225.
32. Pretto, A., L.P. Silva, F.R. Goulart, E.K. Battisti, B.B. Loureiro, D.P. Eggers and D. Pianesso, 2014. [Tung bran in natura or detoxified in the feeding of Hungarian carp (*Cyprinus carpio*) (In Portuguese)]. Braz. Arch. Vet. Med. Anim. Sci., 66: 1891-1900.
33. De Lima, M.R., M. Ludke, F. Neto, B.W.C. Pinto, T.R. Torres and E.J.O. de Souza, 2011. [Mango meal bran for Nile tilapia (In Portuguese)]. Acta Scientiarum Anim. Sci., 33: 65-71.
34. Junior, G.P., P.M. Filho, R. Roubach, P.D.S. Barbosa and E. Shimoda, 2013. [Leucena leaf flour (*Leucaena leucocephala* Lam. De wit) as a protein source for tambaqui juveniles (*Colossoma macropomum* CUVIER, 1818) (In Portuguese)]. Acta Amazonica, 43: 227-234.
35. Tachibana, L., G.S. Goncalves, I.G. Guimaraes, D.R. Falcon, M.M.D. Barros and L.E. Pezzato, 2010. [Replacement of corn by triticale in the feeding of Nile tilapia (In Portuguese)]. Braz. J. Anim. Sci., 39: 241-246.
36. Ranzani-Paiva, M.J. and A.T. Silva-Souza, 2004. [Hematology of Brazilian Fish]. In: Aquatic Health, Ranzani-Paiva, M.J. and R.M. Takemoto and M.A.P. Lizama (Eds.)., Editora Varela, Sao Paulo, Brazil, pp: 89-120.
37. Farhat, K.M.A., 2014. Response of fingerling stinging catfish, *Heteropneustes fossilis* (B loch) to varying levels of dietary l leucine in relation to growth, feed conversion, protein utilization, leucine retention and blood parameters. Aquacult. Nutr., 20: 291-302.
38. Sado, R.Y., D.A.A.J. Bicudo and J.E.P. Cyrino, 2014. Hematology of juvenile pacu, *Piaractus mesopotamicus* (Holmberg, 1887) fed graded levels of Mannan Oligosaccharides (MOS). Latin Am. J. Aquat. Res., 42: 30-39.
39. Soltanzadeh, S., A.E. Fereidouni, H. Ouraji and K.J. Khalili, 2016. Growth performance, body composition, hematological and serum biochemical responses of beluga (*Huso huso*) juveniles to different dietary inclusion levels of faba bean (*Vicia faba*) meal. Aquacult. Int., 24: 395-413.
40. Abdel-Tawwab, M., K.M. Sharafeldin, M.N. Mosaad and N.E. Ismaiel, 2015. Coffee bean in common carp, *Cyprinus carpio* L. diets: Effect on growth performance, biochemical status and resistance to waterborne zinc toxicity. Aquaculture, 448: 207-213.
41. Apun-Molina, J.P., A. Santamaria-Miranda, A. Luna-Gonzales, J.C. Ibarra-Gamez, V. Medina-Alcantar and I. Racotta, 2015. Growth and metabolic responses of whiteleg shrimp *Litopenaeus vannamei* and Nile tilapia *Oreochromis niloticus* in polyculture fed with potential probiotic microorganisms on different schedules. Lat. Am. J. Aquat. Res., 43: 435-445.
42. Polakof, S., S. Panserat, J.L. Soengas and T.W. Moon, 2012. Glucose metabolism in fish: A review. J. Comp. Physiol. B, 182: 1015-1045.
43. Li, S., C. Sang, J. Zhang, N. Chen, Z. Li, P. Jin and X. Huang, 2018. Effects of acute hyperglycemia stress on plasma glucose, glycogen content and expressions of glycogen synthase and phosphorylase in hybrid grouper (*Epinephelus fuscoguttatus*♀× *E. lanceolatus* ♂). Fish Physiol. Biochem., 44: 1185-1196.
44. De Souza, A.M., A.T. Silva, F.E.A. Silva, D.F.B. Campeche, J.F.B. Melo and L.V.O. Vidal, 2019. Mesquite bean (*Prosopis juliflora*) meal in diets of Nile tilapia (*Oreochromis niloticus*): Nutritional value, growth, physiological responses and health. Aquacult. Res., 50: 49-62.
45. Castro, C., G. Corraze, A. Perez-Jimenez, L. Larroquet, M. Cluzeaud, S. Panserat and A. Oliva-Teles, 2015. Dietary carbohydrate and lipid source affect cholesterol metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. Br. J. Nutr., 114: 1143-1156.
46. Ribeiro, P.A.P., L.S. Costa, R.T. Pereira, L.D.S. Murgas and P. Vieira, 2013. [Metabolic parameters of pacus fed with different oil sources]. Braz. Agric. Res., 48: 1035-1042.
47. Hasegawa, M.Y., J.H. Fonteque, A. Kohayagawa and L.P. Boretti, 2002. [Evaluation of the electrophoretic profile of serum proteins in heavy matrices (*Gallus gallus domesticus*) of the Avian farm strain (In Portuguese)]. Rev. Bras. Ciencia Avicola, 4: 203-207.
48. Dallagnol, J.M., L.H. Higuchi, M.L.F. Maluf, A. Feiden and W.R. Boscolo, 2014. [Serum evaluation of Pacus submitted to diets with different levels of protein and energy grown in net tanks]. Acta Iguazu, 3: 97-108.

49. Melo, D.C., D.A.A. Oliveira, M.M. Melo, D.V. Junior, E.A. Teixeira and S.R. Guimaraes, 2009. [Protein profile of chitralized nilotic tilapia (*Oreochromis niloticus*), subjected to chronic stress due to hypoxia (In Portuguese)]. Braz. Arch. Vet. Med. Anim. Sci., 61: 1183-1190.
50. Figueiredo-Silva, A.C., S. Saravanan, J.W. Schrama, S. Panserat, S. Kaushik and I. Geurden, 2013. A comparative study of the metabolic response in rainbow trout and Nile tilapia to changes in dietary macronutrient composition. Br. J. Nutr., 109: 816-826.
51. Beitz, D.C., 1996. [Protein and Amino Acid Metabolism]. In: Physiology of Domestic Animals, Swenson, M.J. and R.W.O. Dukes (Eds.)., Guanabara Koogan, Rio de Janeiro, Brazil, pp: 430-446 (In Portuguese).
52. Bombardelli, R.A., F. Meurer and M.A. Syperreck, 2003. [Protein metabolism in fish (In Portuguese)]. Vet. Sci. Zool. Arch., 7: 69-79.
53. Viegas, I., J. Rito, I. Jarak, S. Leston, A. Caballero-Solares and I. Meton *et al*., 2015. Contribution of dietary starch to hepatic and systemic carbohydrate fluxes in European seabass (*Dicentrarchus labrax* L.). Br. J. Nutr., 113: 1345-1354.
54. Lundsted, L.M., J.F.M. Bibiano and G. Moraes, 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corruscans* (Teleostei: Siluriformes) in response to diet composition. Comp. Biochem. Physiol. Part B, 137: 331-339.
55. Perez-Jimenez, A., G. Cardenete, A.E. Morales, A. Garcia-Alcazar, E. Abellan and M.C. Hidalgo, 2009. Digestive enzymatic profile of *Dentex dentex* and response to different dietary formulations. Comp. Biochem. Physiol. Part A. Mol. Integr. Physiol., 154: 157-164.
56. Couto, A., C. Barroso, I. Guerreiro, P. Pousao-Ferreira and E. Matos *et al*., 2016. Carob seed germ meal in diets for meagre (*Argyrosomus regius*) juveniles: Growth, digestive enzymes, intermediary metabolism, liver and gut histology. Aquaculture, 451: 396-404.
57. Gomez-Requeni, P., M.N. Kraemer and L.F. Canosa, 2012. Regulation of somatic growth and gene expression of the GH-IGF system and PRP-PACAP by dietary lipid level in early juveniles of a teleost fish, the pejerrey (*Odontesthes bonariensis*). J. Comp. Physiol. B., 182: 517-530.