

Mango pulp meal in diets for Nile tilapia (*Oreochromis niloticus*): Digestibility, Growth performance, Biochemical analysis, Digestive enzyme activity, hematological variables

Farinha de polpa de manga em dietas para tilápia do Nilo (Oreochromis niloticus): Digestibilidade, Crescimento; Análise bioquímica; Atividade de enzimas digestivas; variáveis hematológicas

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ABSTRACT

The objective of this study was to evaluate the potential of mango pulp meal (MPM) as an ingredient for extruded diets for Nile tilapia juveniles. In the first trial, the digestibility of energy, dry matter, crude protein, and amino acids was determined, using 80 juveniles (average weight 25.00 ± 0.50 g), fed with a reference diet and a test diet containing the proportion of 70% of the reference diet and 30% of the MPM. Chromium oxide (Cr₂O₆) was used as a digestibility indicator and feces were collected using the Adapted Guelph System. The second assay assessed the growth performance; biochemical analysis; digestive enzyme activity; hematological variables for 300 juveniles fish (average weight 26.66 ± 0.17 g), fed for 45 days with balanced diets containing 0 (control), 40, 80, 120, and 160 g of MPM per kg⁻¹. The dry matter, crude protein, and crude energy apparent digestibility coefficients of MPM were 39.26, 50.18, and 44.5%, respectively. The growth performance of the animals was negatively impacted by the MPM, however the hematological parameters and plasma concentrations of albumin, total proteins and cholesterol were not altered. However, the increase on hepatic glycogen reserves, glycemia, and alanine aminotransferase plasmatic enzyme activity demonstrated that MPM diet inclusion promoted oxidative metabolism conditions that could cause hepatocellular damage and affect fish health, if fed for long periods or at higher levels of MPM than analyzed in this study.

Keywords: energetic ingredient, carbohydrates, agroindustry residues, fish farming.

RESUMO

O objetivo deste estudo foi avaliar o potencial do farelo de polpa de manga (FPM) como ingrediente para dietas extrusadas para juvenis de tilápia do Nilo. No primeiro ensaio, a digestibilidade da energia, matéria seca, proteína bruta e aminoácidos foi determinada, utilizando 80 juvenis (peso médio $25,00 \pm 0,50$ g) alimentados com uma dieta referência e uma dieta teste contendo a proporção de 70% da dieta referência e 30% do FPM. Foi utilizado o óxido de cromo (Cr_2O_6) como indicador de digestibilidade e as fezes foram coletadas por meio do Sistema de Guelph Adaptado. O segundo ensaio avaliou desempenho, análises bioquímicas, atividades de enzimas digestivas e variáveis hematológicas de 300 jovens (peso médio $26,66 \pm 0,17$ g), alimentados com dietas balanceadas contendo 0 (controle), 40, 80, 120 e 160 g de FPM por kg de dieta por 45 dias. Os coeficientes de digestibilidade aparente da matéria seca, proteína bruta e energia bruta do MPM foram 39,26, 50,18 e 44,5%, respectivamente. O desempenho dos animais foi impactado negativamente pela FPM, porém os parâmetros hematológicos e concentrações plasmáticas de albumina, proteínas totais e colesterol não foram alterados. O aumento das reservas hepáticas de glicogênio, glicemia e atividade enzimática plasmática da alanina aminotransferase demonstraram que a inclusão da dieta MPM promoveu condições de metabolismo oxidativo que poderiam causar danos hepatocelulares e afetar a saúde dos peixes, se alimentados por longos períodos ou em níveis mais altos de MPM do que o analisado neste estudo.

Palavras-chave: ingrediente energético, carboidratos, resíduos da agroindústria, piscicultura.

INTRODUCTION

In aquaculture, the possibility of totally or partially replacing traditional ingredients used in diet formulations with alternatives is of great interest. In the evaluation process, the nutritional value of the ingredients must be considered, in addition to the possibility of replacing totally or partially the ingredients of traditional crops (GLENCROSS et al., 2007). Alternative ingredients include non-traditional crop products (DE SOUZA et al., 2018) or even residues from the agribusiness (FELIX E SILVA et al., 2020), enabling the reduction of the impact of activities with large disposal of by-products (SILVA et al., 2018). The fruit agribusiness is an important economic activity in tropical and subtropical countries, and generates large amounts of waste during production and after harvest (MARTINS AND FARIAS, 1994; DURIGAN et al., 2005). Fruits with mechanical or aesthetic damage are often discarded during these phases, and can become a waste product (DE CAMPOS AND DE LIMA, 2014). On the other hand, fruits,

as well as their parts are rich in carbohydrates such as reducers sugar, and pectical substances, which can make them potential substitutes for traditional energy ingredients (KROGDAHL et al., 2005).

In this scenario, mango fruit (*Mangifera indica* L.) has the characteristics to be a possible substitute for traditional energetic ingredients (LAZZARI et al., 2015), with studies carried out with fish and other non-ruminant organisms (VIEIRA et al., 2008; MELO et al., 2012; BEZERRA et al., 2014). In addition to the growth parameters of fish, the use of alternative ingredients can cause changes in animal's health and metabolism. When considering alternative energetic ingredients, due to the change in the carbohydrate matrix, we can observe differences in carbohydrate metabolism, consequently, energy and protein metabolism (DE SOUZA et al., 2018; FELIX E SILVA et al., 2020).

In this context, the purpose of the present study was to determine the apparent digestibility (energy, dry matter, crude protein, carbohydrates and amino acids), growth performance; biochemical analysis;

digestive enzyme activity; hematological variables of juvenile Nile tilapia (*Oreochromis niloticus*), by feeding them diets containing variable levels of mango pulp meal. Thus, to determine the potential use of this residue from the fruit agroindustry in the feeding of Nile tilapia.

MATERIAL AND METHODS

This experiment was performed at the Aquaculture Laboratory of Universidade Federal do Vale do São Francisco – UNIVASF –, Campus Ciências Agrárias, in Petrolina city Pernambuco state. This study was approved by the ethics committee of Universidade Federal da Bahia, process number 54/2015 of CEUA/EMVZ/UFBA. The experiment had two phases. The purpose of the first phase was to evaluate the chemical composition and amino acids profile of mango pulp meal (MPM), and to determine its apparent digestibility coefficients (ADCs) of dry matter (ADCDM), crude protein (ADCCP) and gross energy (ADCGE), and availability of essential amino acids (AEAAs) and non-

essential amino acids (ANEAAAs) for juvenile Nile tilapia. The purpose of the second phase was to determine the growth performance of juvenile tilapia that received diets containing levels of MPM, and the effects of MPM levels on metabolism, intestinal enzymatic activity, and metabolic plasma concentrations.

Manufacture and chemical composition of MPM

Multiple cultivars mangoes fruits, such as Tommy Atkins, unsuitable for human consumption, were collected at the “*Mercado do Produtor*” in Juazeiro/BA. The fruits were processed in a Max Machine pulper, model MDP-150, to separate the epicarp, mesocarp (pulp) and endocarp. Thus, the pulp, the part with the highest concentration of soluble carbohydrates, was dried in a forced ventilation oven (55 ° C) for 48 hours. The dry pulp was ground in a knife mill, using a 1 mm sieve. At the end of the processing, mango pulp meal (MPM) was obtained (Table 1).

Table 1. Analyzed composition of the mango pulp meal

Nutrient	g kg ⁻¹
Dry matter (g kg ⁻¹)	910.00
Ash (g kg ⁻¹) ¹	21.5
Crude protein (g kg ⁻¹)	45.00
Gross energy (kcal kg ⁻¹)	4025.27
Crude fiber (g kg ⁻¹)	99.40
Total phenols (gAT kg ⁻¹)	0.62
<i>Essential amino acids (g kg⁻¹)</i>	
Arginine	5.49
Phenylalanine	1.87
Histidine	0.88
Isoleucine	2.09
Leucine	3.74
Lysine	1.54
Methionine	0.55
Methionine + Cysteine	1.76
Threonine	4.18
Tryptophan	0.99
Valine	2.20
<i>Non-essential amino acids (g kg⁻¹)</i>	
Aspartic acid	3.96
Glutamic acid	4.73
Alanine	4.18
Cysteine	1.21
Glycine	2.31
Proline	2.53
Serine	2.31
Tyrosine	1.65

Digestibility trial

Experimental facilities, animals, and sample collections

Eighty juvenile reversed sex Nile tilapias (25.00 ± 0.50 g) were allocated randomly to eight conical bottom tanks (200 L). Tanks were connected with a recirculation water system consisting of a shared water supply and individual drainage for each tank. The system included, for each tank, a central activated carbon filter with a perlon acrylic cover, and a biological filter with a

reservoir of gravel substrate for bacteria that break down ammonia. Aeration was supplied by a central blower connected to plastic hoses and porous stones supplying each individual tank of experimental fish. Fish were allowed to adapt to tank conditions and experimental diets for four days before fecal sample collections began. The fecal samples were collected into coolers with ice. There were two types of diet: a reference diet (basal), and a test diet composed of 70% of the reference diet (Table 2) and 30% MPM.

Table 2. Chemical composition and amino acid profile reference and test diets

Ingredients	g kg ⁻¹	
Mango pulp meal	0.00	300.00
Poultry by-product meal	305.00	213.50
Wheat middlings	263.8	184.66
Corn	181.00	126.70
Corn gluten meal	130.00	91.00
Soybean meal	106.00	74.20
Mineral and vitamin mixture ^a	5.00	3.50
Dicalcium phosphate	5.00	3.50
Vitamin C ^b	2.00	1.40
Antifungal ^c	1.00	0.70
Chromium Oxide III	1.00	0.70
B.H.T ^d	2.00	1.40
<i>Analyzed chemical composition</i>		
Dry matter	964.00	948.00
Ash	63.16	50.60
Crude protein	418.05	308.44
Gross energy (kcal kg ⁻¹)	4798.76	4863.92
Crude fiber	56.32	69.18
<i>Essential amino acids</i>		
Arginine	22.90	16.98
Phenylalanine	21.20	15.61
Histidine	7.00	5.06
Isoleucine	18.00	13.50
Leucine	42.50	31.22
Lysine	18.90	13.40
Methionine	7.20	4.85
Methionine + Cysteine	13.10	9.81
Threonine	16.30	11.60
Tryptophan	2.80	2.11
Valine	20.70	16.24
<i>Non-essential amino acids</i>		
Aspartic acid	33.70	25.42
Glutamic acid	7.15	52.32
Alanine	33.60	25.11
Cysteine	5.90	4.96
Glycine	27.30	20.46
Proline	31.80	23.73
Serine	22.70	16.03
Tyrosine	13.50	11.71

^aMineral and vitamin mixture: Cobalt (minimum) 80 mg kg⁻¹; Copper (minimum) 3.500 mg kg⁻¹; Iron (minimum) 20 g kg⁻¹; Iodine (minimum) 160 mg kg⁻¹; Manganese (minimum) 10.000 mg kg⁻¹; Selenium (minimum) 100 mg kg⁻¹; (minimum) 24 mg kg⁻¹; Folic acid (minimum) 1200 mg kg⁻¹; Nicotinic acid (minimum) 20 g kg⁻¹; Uric acid (minimum) 10.000 mg kg⁻¹; Biotin (minimum) 200 mg kg⁻¹; Choline (minimum) 100 g kg⁻¹; Inositol (minimum) 25 g kg⁻¹; Vitamin A (minimum) 2.400.000 UI kg⁻¹; Vitamin B1 (minimum) 4.000 mg kg⁻¹; Vitamin B2 (minimum) 4.000 mg kg⁻¹; Vitamin B12 (minimum) 8.000 mg kg⁻¹; Vitamin C (minimum) 60 g kg⁻¹; Vitamin B2 (minimum) 4.000 mg kg⁻¹; Vitamin B6 (minimum) 3.500 mg kg⁻¹; Vitamin D3 (minimum) 600.000

UI kg⁻¹; Vitamin E (minimum) 30.000 UI kg⁻¹; Vitamin K3 (minimum) 3.000 mg kg⁻¹. ^bVitamin C resistant to high pressure and high temperatures, insoluble in water. ^cCalcium propionate. ^dAntioxidant = di-terc-butyl methyl phenol or buzzarded hydroxitolen. ^eAccording to Furuya et al. (2010).

TD composed of 70% of RD and 30% of MPM; Crude protein: Nitrogen x 6.25.

The experimental fish were fed five times a day between 07:00 and 17:00, until they were visibly satiated. One hour after the last feeding of the day, water circulation was closed, each tank was cleaned, and the water was partially changed, while the central blower remained active. Fecal collection tubes, which were individually attached to the bottom each tank, were collected, and stored in coolers with ice. At 0,600 hr the morning after collections, fecal collection tubes were centrifuged. Material in the sediment was saved and other material was discarded. The material was then fine-strained and deposited in plastic bottles labeled for each tank. Feces were pooled between collections into the same bottle for each replicate. These samples were kept frozen at -21°C until the end of the collection period.

Chemical analysis of ingredients, diets, and faeces

After each experimental period, feces were dehydrated in a forced ventilation oven at 55°C for 48 h; the same procedure was applied to the ingredients and the experimental diets, but only for 24 h. All sample materials were then ground in a ball mill.

Dry matter and ash content were measured according to standard methods (AOAC, 2005). Moisture content was determined by drying the samples to a constant weight at 105°C in a furnace (TE-391-1, Tecnal). Nitrogen content was determined using the Micro-Kjeldahl (Tecnal) method, and the crude protein content was estimated by multiplying the nitrogen content by 6.25 (FURUKAWA AND TSUKAHARA, 1966). Lipid content was determined by ether extraction in a multi-unit Soxhlet apparatus (TE-188/6, Tecnal) for 16 h. Ash content was measured by combustion. Samples

were dried in a muffle furnace (TE-1100-1P, Tecnal) at 550°C for 6 h, and crude energy content was assessed using an adiabatic bomb calorimeter (Parr 1266, Parr Instruments Co., Moline, Illinois, USA).

Amino acids were measured commercially by CBO Analysis Laboratory Ltda., Valinhos, São Paulo, Brazil by hydrolyzing a 0.3-mg sample in 1 mL of 6 N HCl for 22 h. The obtained sample was diluted in 0.02 N HCl and injected into an automatic AA analyzer (Hitachi L-888, Tokyo, Japan). Hydrolysis was performed to recover the amino acids using 4-N methanesulfonic acid for tryptophan analysis, and sulfuric acid for amino acid analysis. Chromium (III) oxide (Cr₂O₃) levels were measured using inductively coupled plasma atomic emission spectrophotometry (ICP-AES, Vista-MPX, Varian, Palo Alto, California, USA) after digestion with perchloric acid, following a technique modified from Bezerra Neto and Barreto (2011).

Concentrations of total phenols and tannins in MPM and in experimental diets were determined according to the (AOAC, 2005) acetone extraction method (70%), and colorimetric titration with the Folin-Ciocalteu reagent, sodium carbonate (20%), and insolubilized polyvinylpyrrolidone. Standardized readings were derived from a tannic acid curve (0.1 mg mL⁻¹) and the results were expressed as mg equivalents of tannic acid per kg of dry matter (DM).

Calculation of apparent digestibility coefficients and availability of amino acids

Nutrient and energy ADCs, in addition to AEAAs and ANEAAs, were calculated based on chromium levels in food and feces according equations 1 and 2, following (NRC, 2011):

Equation 1: $ADC_{diet} (\%) = (100 - [chromium\ in\ feed / chromium\ in\ feces] \times [energy\ or\ nutrient\ in\ feces / energy\ or\ nutrient\ in\ feed] \times 100)$
where ADC (n) = apparent digestibility coefficients,

Equation 2:

$ADC\ of\ test\ ingredient (\%) = ADC_{test\ diet} + [(ADC_{test\ diet} - ADC_{reference\ diet}) \times (0.7 \times D_{reference} / 0.3 \times D_{ingredient})]$,

where $D_{reference}$ and $D_{ingredient}$ are the percentages of the nutrients or kcal g⁻¹ gross energy in the reference diet and test ingredients, respectively.

MPM inclusion trial

Fish, experimental conditions, facilities, and diets manufacture

A 45 day trial was conducted to evaluate performance of 300 male, sexually reversed, juvenile Nile tilapia (average weight 26.66 ± 0.17 g) given diets with different concentrations of MPM. The fish were obtained from AAT International Ltda. (Paulo Afonso-BA) and randomly distributed into 20 plastic circular tanks (500 L each). Each tank had a recirculation system with a physical central active carbon filter and a perlon-type acrylic cover. Aeration was supplied to tanks with a central blower connected to plastic hoses leading to porous stones.

Five diet types were tested, containing 0, 40, 80, 120, or 160 g of MPM per kg of diet (Table 3). Each diet type was randomly assigned to each of four tanks. Fish were fed four times a day until visible satiation, at 08,00 hr, 11,00 hr, 14,00 hr, and 17,00 hr.

Table 3. Formulation and proximal composition of trial diets of mango pulp meal for juvenile Nile tilapia.

Ingredients	Mango inclusion (g kg ⁻¹)				
	0 (control)	40	80	120	160
Corn	280.00	280.00	280.00	280.00	280.00
Wheat middlings	255.00	217.00	179.60	141.90	104.20
Poultry by-product meal	200.00	200.00	200.00	200.00	200.00
Corn gluten meal	71.00	71.00	71.00	71.00	71.00
Soybean meal	160.00	160.00	160.00	160.00	160.00
Cellulose	18.00	13.80	9.00	4.50	0.00
DL-Methionine	0.80	3.00	5.20	7.40	9.60
Mineral and vitamin mixture ^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
Vitamin C ^b	2.00	2.00	2.00	2.00	2.00
Calcium Propionate	1.00	1.00	1.00	1.00	1.00
B.H.T ^c	0.20	0.20	0.20	0.20	0.20
Mango pulp meal ^d	0.00	40.00	80.00	120.00	160.00
<i>Nutrients</i>	<i>Calculated proximal composition^e</i>				
Digestible protein (g kg ⁻¹)	261.40	261.00	260.70	260.30	260.00
Ash (g kg ⁻¹)	60.60	58.60	56.70	54.70	52.70
Digestible energy (kcal kg ⁻¹)	3077.17	3061.88	3046.59	3031.30	3016.01
Crude fiber (g kg ⁻¹)	59.30	57.50	55.70	53.80	52.00
Ether extract (g kg ⁻¹)	46.30	46.90	47.50	48.10	48.70
<i>Digestible amino acids (g kg⁻¹)</i>					
Arginine	17.10	16.90	16.70	16.40	16.20
Phenylalanine	12.80	12.70	12.60	12.40	12.30
Histidine	6.40	6.30	6.20	6.10	6.00
Isoleucine	10.10	10.20	10.30	10.50	10.60
Leucine	23.90	23.80	23.60	23.50	23.30
Lysine	16.70	16.60	16.50	16.30	16.20
Methionine + Cysteine	8.00	7.90	7.90	7.80	7.70
Methionine	5.60	7.70	9.80	11.90	14.10
Threonine	8.60	8.60	8.50	8.50	8.40
Tryptophan	3.00	3.00	3.00	2.90	2.90
Valine	12.00	11.90	11.80	1.20	1.20

^aMineral and vitamin mixture: Cobalt (minimum) 80.00 mg kg⁻¹; Copper (minimum) 3,500.00 mg kg⁻¹; Iron (minimum) 20.00 g kg⁻¹; Iodine (minimum) 160.00 mg kg⁻¹; Manganese (minimum) 10,000.00 mg kg⁻¹; Selenium (minimum) 100.00 mg kg⁻¹; (minimum) 24.00 mg kg⁻¹; Folic Acid (minimum) 1200.00 mg kg⁻¹; Nicotinic Acid (minimum) 20.00 g kg⁻¹; Uric Acid (minimum) 10,000.00 mg kg⁻¹; Biotin (minimum) 200.00 mg kg⁻¹; Choline (minimum) 100.00 g kg⁻¹; Inositol (minimum) 25.00 g kg⁻¹; Vitamin A (minimum) 2,400,000.00 UI kg⁻¹; Vitamin B1 (minimum) 4,000.00 mg kg⁻¹; Vitamin B2 (minimum) 4,000.00 mg kg⁻¹; Vitamin B12 (minimum) 8,000.00

mg kg⁻¹; Vitamin C (minimum) 60.00 g kg⁻¹; Vitamin B2 (minimum) 4,000.00 mg kg⁻¹; Vitamin B6 (minimum) 3,500.00 mg kg⁻¹; Vitamin D3 (minimum) 600,000.00 UI kg⁻¹; Vitamin E (minimum) 30,000.00 UI kg⁻¹; Vitamin K3 (minimum) 3,000.00 mg kg⁻¹. ^bVitamin C resistant to high pressures and temperatures. ^cAntioxidants = di-terc-butyl methyl phenol or buzzarded hydroxitolen.. ^dThe MPM digestible energy and nutrients were determined in the first phase of the study. ^eAccording to Furuya et al. (2010)

Water quality parameters (temperature, pH, and dissolved oxygen) were measured daily in the morning and in the afternoon throughout the entire experimental period using a multiparametric digital Alfakit[®] (AT1100) rig.

Growth performance

After the 45 day feeding experiment period, all fish were starved for a 24 hours, and then anesthetized in order to collect blood and biometric data. After this, fish were euthanized and their livers were dissected for further analysis. Data collected on fish included: final weight (FW); final biomass (FB); weight gain ($WG = \text{Final weight} - \text{Initial weight}$); biomass gain ($BG = \text{Final biomass} - \text{Initial biomass}$); hepatic-somatic index ($HSI = 100 \times \frac{[\text{liver weight}]}{[\text{fish weight}]}$); thermal growth coefficient ($TGC = \frac{[(\sqrt[3]{\text{Final weight}} - \sqrt[3]{\text{Initial weight}})]}{(\text{average temperature} \times \text{days})} \times 1000$); feed intake (FI); Feed conversion ratio ($FCR = \frac{\text{feed intake}}{\text{weight gain}}$).

Biochemical analysis

Plasmatic evaluations of metabolic intermediates were made using LABTEST[®] industrial kits for colorimetric determination of glucose (GLUCOSE LIQUIFORM[®]), total proteins (TOTAL PROTEINS[®], BIURETO method) and total cholesterol (CHOLESTEROL LIQUIFORM[®]). Both analyses were conducted with a biochemical semi-automatic veterinary device (Doles[®], model D-250).

Free plasmatic amino acid dosage was measured using the Ninhidrine 0,10% method in propanol, with a standard curve based on a glycine solution of 1.00 millimolar (mM), and read via spectrophotometer wavelength of 570 nanometers (nm).

The plasmatic activity of the enzyme alanine aminotransferase (ALT) was measured with an ALT/GPT Liquiform[®] kit and read with a spectrophotometer with a wavelength of 340 nm at one and three minutes into the reaction. The activity was considered the difference between the two measurements.

Glycogen content of liver tissue was determined following Bidinotto et al. (1997). Liver fragments were weighed and then put in potassium hydroxide (KOH) with 6.00 normal (N). Glycogen contents were extracted with ethanol, centrifuged, and reacted with potassium sulphate (K₂SO₄), water, and phenol. Reaction readings were taken using a spectrophotometer with a wavelength of 480 nm, and compared to the standard curve for glucose, with values in expressed in micromoles per milligram of hepatic tissue (μmol mg⁻¹).

Digestive enzyme activity

For intestinal amylase evaluation, six fish were randomly selected from each treatment and a 3.00 cm portion of intestinal segment was collected from each (before final gastric expansion). Each adipose tissue segment and the rest of the mesentery were taken. Each intestinal fragment was weighed, put in a sample tube with a 2.00 mL buffer solution (tris-phosphate-acid phosphoric-glycerin, pH = 7.00), and macerated using a mechanical tissue refrigerated homogenizer. After tissue

maceration, tubes were centrifuged at 12,000 rpm for three minutes in a refrigerated centrifuge. Then, intestinal amylase enzyme activity was measured using an Amylases Bioclin[®] commercial test kit and a semiautomatic evaluator (Trademark Doles[®], model D-250). Values were expressed as international units of activity in grams of tissue ($U\ g^{-1}$).

Hematological variables

Hematological variables were determined for fresh blood taken from fish tail veins using heparinized syringes. Hematocrits (HT) were measured by a centrifugation method with micro capillary tubes (heparinized in vitro), and compared with a percentage table of micro hematocrits (GOLDENFARB et al., 1971).

To measure hemoglobin values (HB), grams per each deciliter of blood ($g\ dL^{-1}$) (COLLIER, 1944). Corpuscular hemoglobin concentration index (CHCM) was calculated as $CHCM = 100 (HB\ HT^{-1})$, with HT expressed in $g\ dL^{-1}$.

Statistical analysis

Data were tested for normality using Shapiro-Wilk tests, and were analyzed by polynomial regression and linear response plateau (LRP) analysis with the minimums

squared method, according to the model $Y = L + U * (RX)$, where Y = value of the variable analyzed, X = percentage of MPM inclusion, L = plateau response of the studied variable; U = slope of the line, and R = inclusion of MPM estimated by the intercept point (ROBBINS et al., 1979). The Dunnett test was performed to compare the control treatment with the other treatments. Models with the best fit for each variable were chosen. All procedures were conducted the SAS[®] University Edition statistical package.

RESULTS

In both trials, there was no mortality of experimental fish. During the trials, average water temperatures were $26.00 \pm 0.50^{\circ}C$, pH was 6.80 ± 0.40 , and dissolved oxygen was $4.80 \pm 0.30\ mg\ L^{-1}$.

Digestibility Trial

Apparent digestibility of crude energy and crude protein was low, 44.55% and 50.18%, respectively. The average digestibility of amino acids was 56.81%, with a value of 56.28% for the average digestibility of essential amino acids (Table 4).

Table 4. Apparent digestibility and availability coefficients of mango pulp meal.

Nutrients	ADC ¹ (%)
Dry matter	39.26
Crude protein	50.18
Gross energy	44.55
<i>Essential amino acids</i>	
Arginine	39.55
Histidine	60.57
Isoleucine	57.91
Leucine	59.74
Lysine	59.00
Methionine	64.17
Methionine + Cysteine	53.09
Phenylalanine	60.89
Threonine	50.21
Tryptophan	58.10
Valine	55.87
<i>Non-essential amino acids</i>	
Alanine	57.55
Proline	55.72
Cysteine	42.43
Serine	54.00
Tyrosine	64.05
Glycine	44.36
Aspartic acid	70.91
Glutamic acid	71.45

¹Apparent digestibility coefficients of nutrients; ² Availability of essential amino acids; ³ Availability non-essential amino acids.

MPM inclusion trial

All growth performance variables showed differences between treatments with the inclusion of MPM and the control treatment

($p < 0.05$), except for FI and FCR (Table 5). None of the variables fit a model with a biological explanation to the experimental design of the study.

Table 5 Growth performance and efficiency of tilapia fed diets with mango pulp inclusion.

Variables	Inclusion levels for mango pulp meal (g kg ⁻¹)									
	0 (Control)	40		80		120		160		SEM
	Mean	Mean	<i>P</i> <i>value</i>	Mean	<i>P</i> <i>value</i>	Mean	<i>P</i> <i>value</i>	Mean	<i>P</i> <i>value</i>	
FW (g) ¹	89.78	78.53*	0.01	80.09*	0.02	80.35*	0.03	80.50*	0.03	1.26
FB (g) ²	1347	1178*	0.01	1201*	0.02	1205*	0.03	1207*	0.03	18.94
WG (g) ³	63.40	52.00*	0.01	53.49*	0.02	53.38*	0.02	53.68*	0.02	1.29
BG (g) ⁴	951	780*	0.01	802*	0.02	800*	0.02	805*	0.02	19.39
TGC ⁵	1.33	1.15*	0.01	1.18*	0.02	1.17*	0.02	1.18*	0.02	0.02
FI (g) ⁶	1160	1169	1.00	1214	0.76	1212	0.77	1223	0.65	17.11
FCR ⁷	1.24	1.50	0.12	1.52	0.09	1.53	0.07	1.52	0.08	0.04

*Values differ from the control treatment by the Dunnett test ($p > 0.05$). ¹FW = final weight; ²FB = final biomass; ³WG = weight gain; ⁴BG = biomass gain; ⁵TGC = thermal growth coefficient; ⁶FI = Feed intake; ⁷FCR = Feed conversion ratio.

No differences were observed between hematological variables. Among the plasma variables, difference was observed in glucose, AST, ALT and TPAA, as well in hepatic glycogen ($p < 0.05$) (Table 6). Only plasma glucose adjusted to a model with biological explanation (Figure 1).

Table 6. Metabolites, plasma enzymatic activity, and hematology of the Nile tilapia fed diets with mango pulp inclusion.

Variable	Inclusion levels (g kg ⁻¹)									
	0 (Control)	40		80		120		160		SEM
	mean	mean	p value	mean	p value	mean	p value	mean	p value	
<i>Plasma metabolites</i>										
Albumin (g dL ⁻¹)	0.80	0.62	0.31	0.63	0.35	0.69	0.69	0.57	0.13	0.03
Total proteins (g dL ⁻¹)	3.18	2.94	0.74	3.16	1.00	3.03	0.93	2.84	0.47	0.07
Cholesterol (mg dL ⁻¹)	80.81	88.97	0.82	73.79	0.88	94.86	0.43	88.90	0.82	3.21
Glucose (mg dL ⁻¹)	70.07	69.18	1.00	65.27	0.99	101.95	0.08	138.99*	0.01	7.41
<i>Plasma enzymatic activity</i>										
ALT (U L ⁻¹)	7.86	9.38	0.86	14.66*	0.01	14.61*	0.01	11.65	0.22	0.84
AST (U L ⁻¹)	53.12	139.91	0.15	188.63*	0.02	254.51*	0.01	204.08*	0.01	19.40
TPAA (μmol μL ⁻¹)	37.85	37.14	1.00	35.96	0.87	38.59	0.99	45.15*	0.04	1.03
<i>Hematology</i>										
HT (%)	45.05	38.25	0.63	43.75	1.00	45.00	1.00	46.25	1.00	1.19
HB (g dL ⁻¹)	3.54	3.44	0.99	3.18	0.50	3.17	0.49	3.33	0.85	0.08
CHCM (g dL ⁻¹)	7.85	8.61	0.73	6.99	0.64	7.09	0.72	7.37	0.92	0.26
<i>Hepatic tissue</i>										
Total proteins (μmol mg ⁻¹)	2.63	2.39	0.64	2.47	0.88	2.98	0.31	2.78	0.88	0.07
Glycogen (μmol mg ⁻¹) ⁵	53.13	127.98*	0.01	120.32*	0.01	114.24*	0.02	121.60*	0.01	7.55

**Values differ from the control treatment by the Dunnett test ($p > 0.05$).

¹ALT = alanine amino transferase; ²TPAA = total plasmatic amino acids; ³HT = hematocrit; ⁴HB = hemoglobin; ⁴CHCM = corpuscular hemoglobin concentration index;

⁵Glycogen expressed in micro moles of glycosil-glucose for each milligram of hepatic tissue.

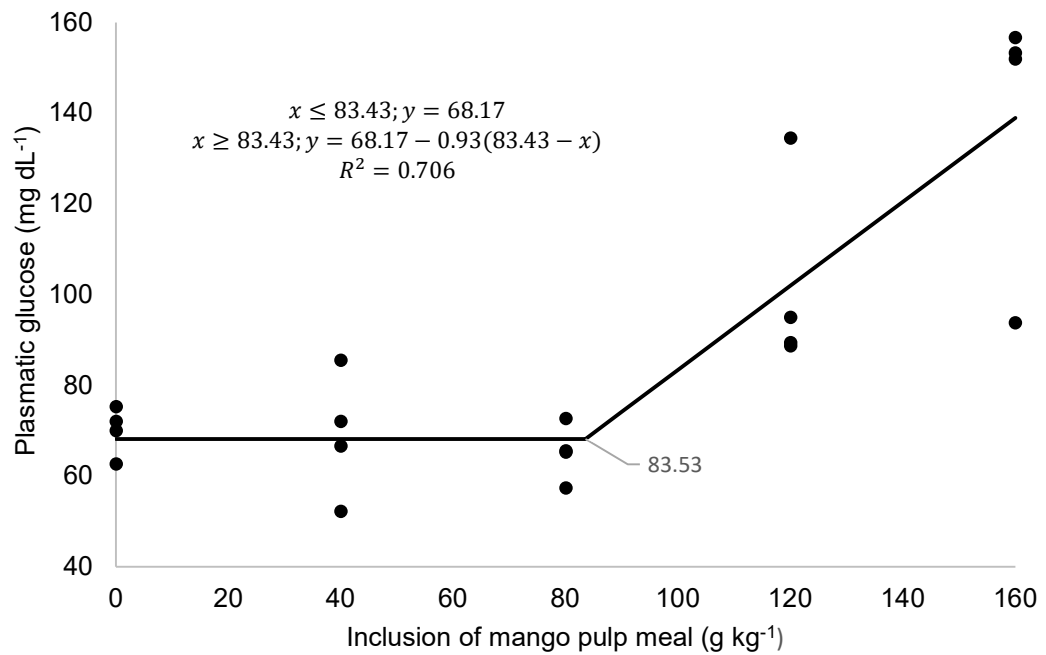


Figure 1. Linear Response Plateau of plasmatic glucose concentrations of Nile tilapia fed diets with different levels of mango pulp meal.

Among digestive enzymes, only amylase activity differed ($p < 0.05$) between the control treatment and the treatments with the inclusion of MPM (Table 7). It was

observed that the amylase activity was reduced until the inclusion of 118.20 g kg⁻¹ of MPF, from that point on, a plateau was observed (Figure 2).

Table 7 Digestive enzymatic activity in Nile tilapia fed diets with mango pulp inclusion.

Variables (U mg ⁻¹ protein)	Inclusion levels (g kg ⁻¹)									
	0 (Control)	40		80		120		160		SEM
	mean	mean	<i>p</i> value	mean	<i>p</i> value	mean	<i>p</i> value	mean	<i>p</i> value	
Amylase	77.95	58.12	0.10	50.27*	0.01	27.82*	0.01	33.49*	0.01	4.22
Lipase	675.60	776.63	0.97	849.08	0.82	1.067.15	0.21	889.73	0.69	65.57
Non-specific protease	2.22	2.02	0.98	2.60	0.82	1.77	0.74	2.36	0.99	0.15

*Values differ from the control treatment by the Dunnett test ($p > 0.05$).

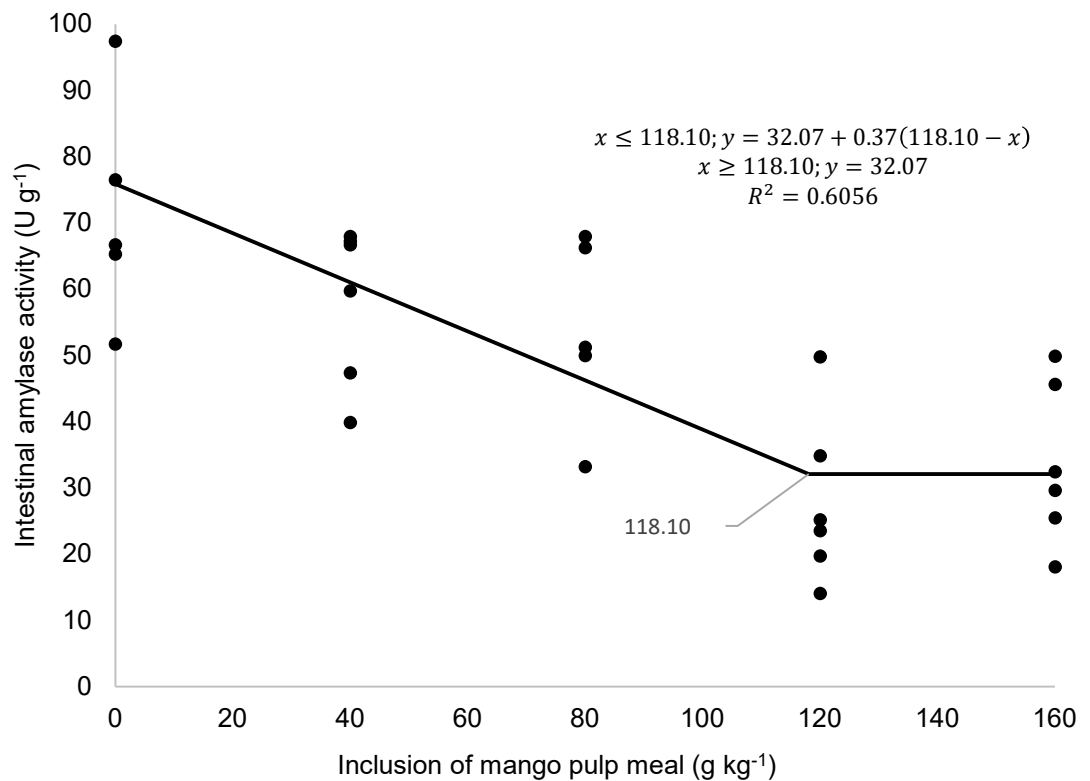


Figure 2. Linear Response Plateau regression of intestinal amylase activity of Nile tilapia fed diets with different levels of mango pulp meal.

DISCUSSION

The levels of dry matter (DM) and crude protein (CP) of the mango pulp meal were similar to those describe for de Lima et al. (2011) for Nile tilapia and Vieira et al. (2008) for poultry, respectively, with DM = 941.00 g kg⁻¹ and CP = 44.40 g kg⁻¹ and DM = 922.30 g kg⁻¹ and CP = 38.70 g kg⁻¹, by studying the composition of industrial solid residues from the fruit pulp industry, containing fruit peels and seeds. Concentrations of crude fiber (CF) observed were different from the actual level of CF = 149.90 g kg⁻¹ and GE = 3.724 kcal kg⁻¹ in the study de Lima et al. (2011), and 146.00 g kg⁻¹ and 3.906 kcal kg⁻¹ in the study by Vieira et al. (2008). Digestibility coefficients for MS, PB, and EB of MPM in our study were lower than those described by de Lima et al. (2011), , who reported values of 78.00, 87.80 and 77.50% respectively. This was likely because de Lima et al. (2011) worked with residues lacking soluble fruit pulp extract. According to Lazzari et al. (2015), mango pulp contains carbohydrates in the forms of fructose, cornstarch, and pectins. These molecules store high amounts of energy in chemical bonds (VERGARA-VALENCIA et al., 2007). However, pectin is a non-starchy carbohydrate and has low digestibility because it is resistant to breakdown by enzymatic hydrolysis or heat treatment (OLIVEIRA et al., 2007).

Although Nile tilapia are omnivorous and capable of consuming pectins and other non-starchy carbohydrates normally included in their diet (KROGDAHL et al., 2005), such

carbohydrates can adversely affect hydrolysis of proteins. This can occur even in fish species such as Nile tilapia (KROGDAHL et al., 2005), which have active β -galactosidase (type of pectin) in their anterior and middle intestines (TANIGUCHI AND TAKANO, 2004). Furthermore, Irvin et al. (2016), described negative effects of pectin on digestibility of energy and nutrients in barramundi fish (*Lates calcarifer*), and pectin could also affect the viscosity and transit velocity in intestines. These results are supported by those of Castillo and Gatlin (2015), who found that addition of exogenous carbohydrase to the diet of juvenile tilapia increased the digestibility of vegetable ingredients. Therefore, compared with pulp residues, we would expect that MPM has lower digestibility of nutrients and energy, and lower availability of amino acids, because of greater interference from soluble fibers that are indigestible, such as pectin.

Increasing concentrations of MPM lead to a significant performance changes ($p > 0.05$) in the juvenile tilapia tested during our study, results are not consistent with those of de Lima et al. (2011) and Melo et al. (2012). Although both of those studies used juvenile tilapia, they were conducted using agro-industrial residues resulting from mango pulp meal processing. These two studies did not detect any performance decrease with an inclusion of a maximum 150 or 300 g MPM per kg of diet, respectively. In contrast, Souza et al. (2013), who tested MPM that included peels, observed a reduction in performance of Nile tilapia with increasing MPM

concentrations. Therefore, we advise against inclusion of MPM levels over 100 g kg^{-1} because of the harmful effects of phenols, such as tannins contained in peels, even though this possibility was not directly assessed in our study.

Melo et al. (2012) replaced corn bran in juvenile Nile tilapia diets with variable levels of mango pulp (0, 33, 66, or 100%) and did not observe any effect of mango pulp on feed intake or FCR, as we did in our study. However, Souza et al. (2013), observed a negative effect on feed intake and FCR of a MPM including peels, at concentrations of over 200 g of MPM per kg of diet. This negative effect may have been due to phenolic contents of fruit peels that were incorporated in the MPM. Pinto et al. (2001) reported negative effects of tannins on the digestion process of juvenile tilapia, for concentrations equal to or higher than 6 g kg^{-1} . These concentrations were much higher than the ones in our study (total phenols of 0.62 gAT kg^{-1}).

According to Rosset et al. (2016), ingestion of large quantities of fructose leads to an increase in the production of metabolic intermediates involved in fructolysis, the process of converting fructose to glucose, lactate, glycerol, and fatty acids, or for use in oxidative metabolism in mitochondria. A similar effect was found in our study, and by Bezerra et al. (2014), who tested levels of 200, 300, 400, and 500 g kg^{-1} of MPM in the diet of the tambaqui fish (*Colossoma macropomum*). That study reported changes in glycogen hepatic reserves corresponding to the level of MPM inclusion in diets. However, results of de Lima et al. (2011), who incorporated levels of 0, 50, 100, and 150 g kg^{-1} of MPM in the tilapia diet, differed from results in our study in that they did

not observe an effect of MPM on glycogen reserves. These contrasting results might have occurred because the type of mango residue used by de Lima et al. (2011) had lower amounts of fructose, which was removed by a fruit processing step.

Amylase, a pancreatic enzyme secreted in the intestine, is responsible for hydrolysis of polysaccharides. Its activity is related to the utilization of different levels of starchy carbohydrates in food, and varies according to feeding routines of species (STECH et al., 2009). The activity of plasmatic ALT increased with the inclusion MPM. this enzyme is present in several tissues of the body, especially hepatocytes. Elevation in plasma ALT is associated with increased permeability of cytoplasmic membranes, oxidative stress, and metabolic imbalance (JONSSON et al., 2002; KOBAYASHI et al., 2009).

According to Rosset et al. (2016), fructose absorption has no regulation system like that of glucose. Hence, ingestion of large quantities of fructose can lead to a cytoplasmic increase in fructose concentration. Fructose would then be phosphorylated, leading to depletion of hepatic adenosine triphosphate (ATP) reserves, and an increase in the turnover of proteins, especially those involved in deamination of amino acids for energy purposes. Higher fructose levels in diets would reduce transamination of amino acids for energy production, leading to what Boscolo et al. (2011) described as a protein-saving effect of carbohydrates. In a subsequent study, higher concentrations of fructose increased the activity of ALT, leading to a pro-oxidative effect when 100 g kg^{-1} lyophilized mango pulp meal was

included in the diet of rats (TOLEDO et al., 2013). This may have been because the lyophilized mango pulp meal led to a mobilization of amino acids in order to keep appropriate glucose levels in blood and to preserve hepatic glycogen reserves through gluconeogenesis. This side effect was also noted by (BEZERRA et al., 2014), who tested mango pulp levels (ranging from 200 to 500 g kg⁻¹) in the diet of tambaquis. High levels of fructose in diets containing MPM can cause oxidative damage to hematocrits and increase plasmatic activity of enzymes similar to ALT. However, MPM levels did not influence hematologic variables. Akrami et al. (2013), researched the prebiotic effect of polymeric carbohydrates of fructose (fruitoligosaccharides) in a sturgeon species (*Acipenser stellatus*), and only observed an increase in average levels of white blood cells. They did not report any significant differences in the hematimetric index.

The effect of including MPM on digestive enzyme activity was similar to that observed when replacing corn with mesquite bran in diets for Nile tilapia. Some enzymes have their production related to the amount of the substrate that catalyzes, thus, the lower proportion of starch induces less amylase production and directs the body's efforts to produce other carbohydrases (DE SOUZA et al., 2018).

In our study, we prepared isoproteic and isoenergetic diets in order to control for carbohydrate compositions of MPM. Consequently, there none of the risk factors described above could have affected the hematologic variables we analyzed. We found that the inclusion of MPM in diets for Nile tilapia reduced the parameters of fish growth, on the other

hand, the hematological parameters do not indicate a significant impact on health in the evaluated period. Furthermore, our data concerning metabolism indicated that inclusion of 54.14 g kg⁻¹ or greater concentrations resulted in metabolism changes in proteins. Therefore, future research to evaluate metabolic and cellular damage by long-term ingestion of MPM could be valuable.

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REFERENCES

- AKRAMI, R.; IRI, Y.; KHOSHBAVAR ROSTAMI, H.; RAZEGHI MANSOUR, M. Effect of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival, lactobacillus bacterial population and hemato-immunological parameters of stellate sturgeon (*Acipenser stellatus*) juvenile. **Fish & Shellfish Immunology**, v. 35, n. 4, p. 1235-1239, 2013.
- AOAC. **Official methods of analysis of AOAC International**. Gaithersburg, Md.: AOAC International, 2005. ISBN 1080-0344 0935584757 9780935584752.
- BEZERRA NETO, E.; BARRETO, L.P. **Análises químicas e bioquímicas em plantas**. Editora Universitária da UFRPE, 2011. 261
- BEZERRA, S.K.; SOUZA, R.C.; MELO, J.F.B.; CAMPECHE, D.F.B.

Crescimento de tambaqui alimentado com diferentes níveis de farinha de manga e proteína na ração. **Archivos de Zootecnia**, v. 63, n. 244, p. 587-598, 2014.

BIDINOTTO, P.M.; MORAES, G.; SOUZA, R.H.S. Hepatic glycogen and glucose in gigh tropical freshwater teleost fish: A procedure for field determinations of micro samples. **Boletim Técnico CEPTA**, p. 53-60, 1997.

BOSCOLO, W.R.; SIGNOR, A.; FREITAS, J.M.A.D.; BITTENCOURT, F.; FEIDEN, A. Nutrição de peixes nativos. **Revista Brasileira de Zootecnia**, v. 40, p. 145-154, 2011.

CASTILLO, S.; GATLIN, D.M. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. **Aquaculture**, v. 435, p. 286-292, 2015.

COLLIER, H.B. Standardization of Blood Haemoglobin Determinations. **Canadian Medical Association journal**, v. 50, n. 6, p. 550-2, 1944.

DE CAMPOS, R.F.F.; DE LIMA, C. Sustentabilidade através de remanejamento de resíduos com prática de gestão ambiental implantado no supermercado cereal. **Ignis: Periódico Científico de Arquitetura e Urbanismo, Engenharias e Tecnologia da Informação**, v. 3, n. 1, p. 25-44, 2014.

DE LIMA, M.R.; LUDKE, M.D.C.M.M.; PORTO NETO, F.D.F.; PINTO, B.W.C.; TORRES, T.R.; DE SOUZA, E.J.O. Farelo de resíduo de manga para tilápia do Nilo. **Acta**

Scientiarum. Animal Sciences, v. 33, n. 1, p. 65-71, 2011.

DE SOUZA, A.M.; SILVA, A.T.; FELIX E SILVA, A.; CAMPECHE, D.F.B.; MELO, J.F.B.; VIDAL, L.V.O. Mesquite bean (*Prosopis juliflora*) meal in diets of Nile tilapia (*Oreochromis niloticus*): Nutritional value, growth, physiological responses and health. **Aquaculture Research**, n. May, p. 1-14, 2018.

DURIGAN, M.F.B.; MATTIUZ, B.-H.; DURIGAN, J.F. Injúrias mecânicas na qualidade pós-colheita de lima ácida 'Tahiti' armazenada sob condição ambiente. **Revista Brasileira de Fruticultura**, v. 27, n. 3, p. 369-372, 2005.

FELIX E SILVA, A.; COPATTI, C.E.; DE OLIVEIRA, E.P.; BONFÁ, H.C.; MELO, F.V.S.T.D.; CAMARGO, A.C.D.S.; MELO, J.F.B. Effects of whole banana meal inclusion as replacement for corn meal on digestibility, growth performance, haematological and biochemical variables in practical diets for tambaqui juveniles (*Colossoma macropomum*). **Aquaculture Reports**, v. 17, n. September 2019, p. 100307-100307, 2020.

FURUKAWA, A.; TSUKAHARA, H. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. **Nippon Suisan Gakkaishi**, v. 32, n. 6, p. 502-506, 1966.

FURUYA, W.M.; PEZZATO, L.E.; BARROS, M.M.; BOSCOLO, W.R.;

CYRINO, J.E.P.; FURUYA, V.R.B.; FEIDEN, A. **Tabelas Brasileiras para a nutrição de tilápias**. 1. Toledo: GFM Gráfica & Editora, 2010. 100-100

GLENCROSS, B.D.; BOOTH, M.; ALLAN, G.L. A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. **Aquaculture Nutrition**, v. 13, n. 1, p. 17-34, 2007.

GOLDENFARB, P.B.; BOWYER, F.P.; HALL, E.; BROSIUS, E. Reproducibility in the hematology laboratory: the microhematocrit determination. **American journal of clinical pathology**, v. 56, n. 1, p. 35-9, 1971.

IRVIN, S.; BLYTH, D.; BOURNE, N.; GLENCROSS, B. A study of the discrete and interactive effects of different polysaccharides on the digestibility of diets fed to barramundi (*Lates calcarifer*). **Aquaculture Nutrition**, v. 22, n. 5, p. 1047-1054, 2016.

JONSSON, C.M.; FERRACINI, V.L.; PARAÍBA, L.C.; RANGEL, M.; AGUIAR, S.R. Alterações bioquímicas e acúmulo em pacus (*Metynnis argenteus*) expostos ao paclotrazol. **Scientia Agricola**, v. 59, p. 441-446, 2002.

KOBAYASHI, A.; SUZUKI, Y.; KUNO, H.; SUGAI, S.; SAKAKIBARA, H.; SHIMOI, K. Effects of fenofibrate on plasma and hepatic transaminase activities and hepatic transaminase gene expression in rats. **The Journal of Toxicological Sciences**, v. 34, n. 4, p. 377-387, 2009.

KROGDAHL, A.; HEMRE, G.I.; MOMMSEN, T.P. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. **Aquaculture Nutrition**, v. 11, n. 2, p. 103-122, 2005.

LAZZARI, R.; UCZAY, J.; RODRIGUES, R.B.; PIANESSO, D.; ADORIAN, T.J.; MOMBACH, P.I. Utilização de resíduos de frutas em dietas para piava. **Boletim do Instituto de Pesca**, v. 41, n. 2, p. 227-237, 2015.

MARTINS, C.R.; FARIAS, R.D.M. **Revista da Faculdade de Zootecnia, Veterinária e Agronomia**. Pontifícia Universidade Católica do Rio Grande do Sul, 1994.

MELO, J.F.B.; SEABRA, A.G.L.; SOUZA, S.A.; SOUZA, R.C.; FIGUEIREDO, R.A.C.R. Substituição do farelo de milho pela farinha de manga no desempenho da tilápia-do-nylo. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 64, n. 1, p. 177-182, 2012.

NRC. **Nutrient Requirements of fish and shrimp**. Washington: The National Academy Press, 2011. 376-376

OLIVEIRA, G.R.D.; LOGATO, P.V.R.; FREITAS, R.T.F.D.; RODRIGUES, P.B.; FIALHO, E.T.; DIODATTI, F.C. Digestibilidade de nutrientes em ração com complexo enzimático para tilápia-do-nylo. **Revista Brasileira de Zootecnia**, v. 36, n. 6, p. 1945-1952, 2007.

PINTO, L.G.Q.; PEZZATO, L.E.; MIRANDA, E.C.D.; BARROS, M.M.

Desempenho do piauçu (*Leporinus macrocephalus*) arraçoado com dietas contendo diferentes teores de tanino. **Revista Brasileira de Zootecnia**, v. 30, n. 4, p. 1164-1171, 2001.

ROBBINS, K.R.; NORTON, H.W.; BAKER, D.H. Estimation of nutrient requirements from growth data. **The Journal of nutrition**, v. 109, n. 10, p. 1710-4, 1979.

ROSSET, R.; SUROWSKA, A.; TAPPY, L. Pathogenesis of Cardiovascular and Metabolic Diseases: Are Fructose-Containing Sugars More Involved Than Other Dietary Calories? **Current Hypertension Reports**, v. 18, n. 6, p. 44-44, 2016.

SILVA, C.B.; VALENTE, L.M.P.; MATOS, E.; BRANDÃO, M.; NETO, B. Life cycle assessment of aquafeed ingredients. **International Journal of Life Cycle Assessment**, v. 23, n. 5, p. 995-1017, 2018.

SOUZA, R.C.; MELO, J.F.B.; NOGUEIRA FILHO, R.M.; CAMPECHE, D.F.B.; FIGUEIREDO, R.A.C.R. Influencia da farinha de manga no crescimento e composição corporal da tilápia do Nilo. **Archivos de Zootecnia**, v. 62, n. 238, p. 217-225, 2013.

STECH, M.R.; CARNEIRO, D.J.; JÚNIOR, J.M.P. Fatores que afetam a produção de enzimas digestivas em peixes e o uso de enzimas exógenas como ferramentas em nutrição de peixes. **Ensaios e Ciências Biológicas, Agrárias e da Saúde**, v. 13, n. 2, p. 79-93, 2009.

TANIGUCHI, A.Y.; TAKANO, K. Purification and properties of beta-galactosidase from Tilapia intestine: Digestive enzyme of Tilapia-X. **Fisheries Science**, v. 70, n. 4, p. 688-694, 2004.

TOLEDO, R.C.L.; BRITO, L.F.D.; RIBEIRO, S.M.R.; PELUZIO, M.D.C.G.; SIQUEIRA, C.L.M.D.; QUEIROZ, J.H.D. Efeito da ingestão da polpa de manga (*Mangifera indica* L.) sobre os parâmetros bioquímicos séricos e integridade hepática em ratos. **Bioscience Journal**, v. 29, n. 2, 2013.

VERGARA-VALENCIA, N.; GRANADOS-PÉREZ, E.; AGAMA-ACEVEDO, E.; TOVAR, J.; RUALES, J.; BELLO-PÉREZ, L.A. Fibre concentrate from mango fruit: Characterization, associated antioxidant capacity and application as a bakery product ingredient. **LWT - Food Science and Technology**, v. 40, n. 4, p. 722-729, 2007.

VIEIRA, P.A.F.; QUEIROZ, J.H.D.; ALBINO, L.F.T.; MORAES, G.H.K.D.; BARBOSA, A.D.A.; MÜLLER, E.S.; VIANA, M.T.D.S. Efeitos da inclusão de farelo do resíduo de manga no desempenho de frangos de corte de 1 a 42 dias. **Revista Brasileira de Zootecnia**, v. 37, n. 12, p. 2173-2178, 2008.