

INCLUSION OF MESQUITE POD MEAL (*Prosopis juliflora*) IN DIETS FOR NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

Analene Alves Nascimento¹
José Fernando Bibiano Melo¹
Anderson Miranda de Souza²
Fúlvio Viegas Santos Teixeira Melo³

¹Federal University of Vale do São Francisco - UNIVASF Agricultural Sciences Campus. Rodovia BR 407, km 12, Lot 543, Nilo Coelho Irrigation Project, CEP: 56300-000. Petrolina, PE, Brazil. E-mail: analenealves@hotmail.com (autor correspondente).

²Federal University of Western Bahia, UFOB, Barra Multidisciplinary Center, Av. 23 de Agosto, 872, CEP 47100-000, Barra - BA, Brazil.

³Federal Institute of Education Science and Technology, Catu Campus - BA, Rua Barão de Camaçari, 110, CEP 48110-000, Catu - BA, Brazil.

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ABSTRACT

Alternative feeds are used to include or replace conventional ingredients in fish diets to generate regional socioeconomic sustainability and maintain the growth performance of fish. The objective of this study was to evaluate the effect of mesquite pod meal (MPM) in extruded diets for Nile tilapia juveniles fed graded levels of inclusion (26, 32, 38, 44, and 50%). Growth performance variables of plasma metabolites, enzymatic activities and haematological parameters were evaluated. Fish were fed at 6% of biomass for 45 days. Four-hundred Nile tilapia juveniles (11.5 ± 0.18 g) were distributed into twenty-500L aquaria in recirculation system. Significant differences of treatments on performance parameters, feed conversion ratio and survival were observed. Among the metabolic parameters, glycemia was altered ($P < 0.05$). The activities of digestive and transamination enzymes were not influenced ($P > 0.05$) by the inclusion of MPM. Hematological variables did not change ($P > 0.05$). It was concluded that MPM can be included up to 38.67% in diets for Nile tilapia juveniles without impairing performance, metabolism, enzymatic activities and health.

Key words: alternative feeds; aquaculture; fish nutrition.

INCLUSÃO DO FARELO DA VAGEM DA ALGAROBA (*Prosopis juliflora*) NA ALIMENTAÇÃO DE JUVENIS DA TILÁPIA DO NILO (*Oreochromis niloticus*)

RESUMO

Alimentos alternativos são usados para incluir ou para substituir ingredientes convencionais em dietas para peixes. Eles podem gerar sustentabilidade socioeconômica regional e manter os índices produtivos dos animais. Desse modo, objetivou-se avaliar o efeito do farelo da vagem da algaroba (FVA) em dietas extrusadas para juvenis de tilápia do Nilo, com diferentes níveis de inclusão (26, 32, 38, 44, e 50%). Foram avaliadas as variáveis zootécnicas, metabólitos plasmáticos, atividades enzimáticas e parâmetros hematológicos. A taxa de arraçoamento foi de 6% da biomassa no decorrer de 45 dias. Foram distribuídos 400 juvenis ($11,5 \pm 0,18$ g), distribuídos em 20 caixas de 500L, num sistema de recirculação de água com biofiltro. Foram observadas diferenças significativas entre os tratamentos em relação aos parâmetros de desempenho avaliados, com exceção da conversão alimentar aparente e sobrevivência. Dentre os parâmetros metabólicos, a glicemia foi alterada ($P < 0,05$). As atividades das enzimas digestivas e de transaminação não tiveram influência da inclusão do FVA ($P > 0,05$). As variáveis hematológicas não sofreram alterações ($P > 0,05$). O FVA pode ser incluído em dietas para juvenis de tilápia do Nilo em até 38,67% sem prejudicar o desempenho, o metabolismo, as atividades das enzimas e a saúde desta espécie.

Palavras-chave: alimentos alternativos; aquicultura; nutrição de peixes.

INTRODUCTION

Alternative feeds are included in fish diets to reduce feed costs and generate regional socioeconomic sustainability (Bicudo et al., 2018). Fava beans (Azaza et al., 2009), coffee residues (Pimenta et al., 2011), mango residue (Melo et al., 2012; Souza et al., 2013), peanut shells (Pessoa et al., 2013), cassava leaves and mesquite pod meal (Jesus et al., 2011), have demonstrated potential usefulness. In order for an alternative feed to be considered industrially viable, it must be low cost, high volume of production, regionally or nationally available and increase growth performance of fish (Glencross et al., 2007). In addition, the importance of the extensive knowledge of nutritional characteristics, nutrient digestibility and evaluation of the presence of antinutritional

factors are of fundamental importance (Boscolo et al., 2011; Hisano et al., 2013; Bosisio et al., 2017).

Among the alternative energy sources, it is possible to use mesquite (*Prosopis juliflora*) pod meal, was originated from Peru and was introduced in Brazil in the Northeast region to promote reforestation and animal feed (Damasceno et al., 2017). Mesquite pod meal (MPM) is the main co-product commonly used for consumption, is used in feeds for cattle, goats and sheep (Almeida et al., 2017). Recently, pod meal has been proved as nutritive source of energy and protein (Braga et al., 2010) and amino acids (Souza et al., 2018) for Nile tilapia.

Whole mesquite pod meal possesses a similar chemical composition of maize (Oliveira et al., 2010), also demonstrates high protein and energy digestibility for Nile tilapia (Silva et al., 2015), may be included up to 20% in diets for Nile tilapia juveniles without compromising growth performance (Jesus et al., 2011; Sena et al., 2012). Therefore, the objective of this study was to evaluate the effects of graded levels of mesquite pod meal inclusion on growth performance, metabolites, digestive and transamination enzymes, as well as the hematological parameters of Nile tilapia juveniles.

MATERIAL AND METHODS

This study was previously approved by the Federal University of the São Francisco Valley Ethics Committee on the Use of Animals (License No. 0007/010617).

Experimental design

Experimental diets were formulated to contain 26, 32, 38, 44, and 50% inclusion of the MPM for Nile tilapia juveniles. Four-hundred masculinized Nile tilapia juveniles ($11.5 \pm 0.18\text{g}$) were distributed in 20 circular aquaria of 500 L each, in recirculation system at $1.4 \text{ L}\cdot\text{min}^{-1}$, each experimental unit contained 20 fish. Fish were acclimated to the experimental conditions for a period of seven days, and after this period they received the experimental diets at a level of 6% of the live weight for biometric measurements were performed every 15 days to determine their

weight and adjust the amount of feed provided and to monitor the growth of the animals.

The feeding frequency of three times a day (8:00 AM, 12:00 PM and 4:00 PM) was adopted. Daily, the walls of the boxes were cleaned, and the experimental units were siphoned to remove 20% of the water and to remove any leftovers of diets and feces. The water condition in the experimental units was maintained with the mean values for temperature ($27.0 \pm 1.0^\circ\text{C}$), dissolved oxygen ($7.1 \pm 0.6 \text{ mg L}^{-1}$) and pH (7.5 ± 0.8) within the ranges considered ideal for the development of the species according to Workagegn (2012).

Formulation and preparation of experimental diets

The mesquite pod meal was commercially obtained, packed in plastic bags for the execution of the diets of the experimental test. The formulations of the experimental diets were elaborated with the aid of the SUPER CRAC® computer program based on nutritional information available for tilapia. The ingredients were ground in a hammer-type grinder in 1 mm sifters, subsequently mixed according to the formulations. The feed extrusion was carried out by pre-moistening the mixture with water at a temperature of 100°C using a commercial extruder. After extrusion, the material was oven dried at 55°C for 24 hours, then packed in identified plastic bags and stored in a freezer at -20°C until the time of delivery to the animals.

Experimental diets were analyzed for chemical composition according to the standards of the Association of Official Analytical Chemists (AOAC, 2005). Dry matter was determined by drying the samples to a constant weight at 105°C in a forced ventilation oven. The mineral matter was determined in a muffle furnace at 550°C for 24 hours. The nitrogen content was determined using a Micro-Kjeldahl (Tecnal), and crude protein content was estimated by multiplying the nitrogen content by 6.25. The gross energy was determined with a calorimetric pump.

Five experimental diets, isoproteic, isoenergetic and isofibrous were formulated according to the nutritional requirements of juveniles of the species, with increasing levels of inclusion of mesquite pod meal 26 the 50%, and the chemical composition previously calculated and analyzed (Table 1).

Table 1. Formulation and chemical composition calculated and analyzed of diets containing different levels of inclusion of mesquite pod meal for Nile tilapia juveniles.

	Inclusion levels of mesquite pod meal (in percentage)				
	26	32	38	44	50
60% Corn gluten meal	15.00	15.00	15.00	15.00	15.00
45% Soy meal	16.76	16.40	15.88	15.44	15.00
Poultry viscera flour	9.00	9.00	9.00	9.00	9.00
Mesquite meal	26.00	32.00	38.00	44.00	50.00
Corn meal	14.82	11.30	7.96	4.53	1.09
Wheat flour	8.35	6.44	4.51	2.60	0.69
Soy oil	1.00	0.88	0.76	0.64	0.52

α -Cellulose	2.49	2.16	1.82	1.47	1.13
Dicalcium phosphate	0.50	0.50	0.50	0.50	0.50
L-Lysine- HCL	0.45	0.56	0.67	0.79	0.90
DL-Methionine	0.46	0.60	0.73	0.87	1.00
Mineral and vitamin mix ¹	5.00	5.00	5.00	5.00	5.00
Ascorbic acid ²	0.05	0.05	0.05	0.05	0.05
Antifungal ³	0.10	0.10	0.10	0.10	0.10
BHT ⁴	0.02	0.02	0.02	0.02	0.02
Calculated ⁵ and analyzed ⁶ chemical composition					
Crude protein (%) ⁶	32.44	32.74	32.60	32.69	32.55
Digestible protein (%) ⁵	26.00	26.00	26.00	26.00	26.00
Gross energy (kcal. kg-1) ⁶	4221	4180	4218	4276	4441
Digestible energy (kcal. kg-1) ⁵	3395	3446	3497	3473	3600
Total carbohydrates (%) ⁵	52.87	52.47	52.16	51.81	51.45
Extrato etéreo (%) ⁵	4.50	4.32	4.14	3.95	3.76
Crude fiber (%) ⁵	4.96	4.95	4.95	4.94	4.93
Dry matter (%) ⁶	90.09	90.51	90.37	90.54	90.55
Mineral material (%) ⁶	3.11	3.03	2.95	2.87	2.78

¹Mineral and vitamin mixtures for fish - chemical composition per kilogram of product: A, 1,200.00IU; D, 200.00IU; E 12,000mg; K3, 2400mg; B1, 4800mg; B2, 4800mg; B6, 4000mg; B12, 4800mg; Folic Acid, 1200mg; Ca pantothenate, 12,000mg; C, 48,000mg; Biotin, 48mg; Choline, 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 resistant to high pressures and temperatures and insoluble in water. ³Calcium propionate. ⁴Antioxidant = di-tert-butyl methylphenol or butylated hydroxytoluene. ⁵According to Furuya et al. (2010).

Growth performance

Growth performance data were: Final body weight (FBW = final weight - initial weight/ total days of the experiment); feed intake (FI = total feed consumed/ number of fish per replicate); feed conversion ratio (FCR = total feed intake/ weight gain) and survival rate (SUR = 100 × final fish number/ initial fish number).

Biological material and metabolic profile

At the end of the experimental period, twelve animals of each treatment were sampled for blood collection through puncturing the caudal vessel with syringes containing 10% EDTA. The fish were anesthetized with benzocaine (1g 10L⁻¹), then blood samples were collected; soon after, the fish were euthanized to remove their livers, intestines and muscles, and were kept at a temperature of -20 °C.

Blood was used for the analysis of the metabolites, then the plasma was obtained by centrifugation at 5,000 x rpm for 5 minutes or 2,300 G. Subsequently, all biological material was stored at -20 °C. Total plasma (g dL⁻¹), serum albumin, total cholesterol and triglycerides (mg dL⁻¹) were measured in plasma using commercial biochemical test kits (Labtest®) with colorimetric measurements. Free plasma amino acids were determined according to Copley (1941) by the 0.1% Ninhydrin in propanol method, with a standard curve of 1mM glycine solution read in a spectrophotometer at 570 nm.

Hepatic and muscle glycogen was quantified as described by Bidinotto et al. (1997). Samples of each fish (liver and muscle) were weighed in a ratio of close to 100 mg and transferred to test tubes. Then 1.0 mL of 6.0 N KOH was added and placed for 3 minutes in a water bath at 100 °C to dissolve the entire tissue. Subsequently, 100 μ L of this dissolved extract was transferred to a tube and 250 μ L of ethanol and 100 μ L of 10% K₂SO₄ were added. The sample was centrifuged at 3,000 rpm for 3 minutes. Subsequently, the supernatant was discarded, and the precipitate resuspended in 2 mL of distilled water. After mixing, 100 μ L of the sample, 250 μ L phenol and 1 mL H₂SO₄ were transferred to inhibit the reaction. A volume close to 2 mL of this solution was analyzed for its total reducing sugars content by the hydrolytic method of Dubois et al. (1956). The glycogen content was measured in a spectrophotometer with a wavelength of 480 nm, with values expressed in micromoles (μ M) of glucose per mg of tissue.

Activity of digestive and transaminase enzymes

To determine digestive enzyme activities, the tissues were homogenized in buffer (10 mM phosphate/ 20 mM Tris-pH 7.0) for one minute (4 °C) using a homogenizer. Then the samples were centrifuged at 5000 rpm for five minutes, and shortly after the supernatants were used in the enzymatic assays.

The amylase activity was estimated using the commercial Labtest® kit. The sample was incubated with a starch substrate, after iodine was added, it was compared to a control, then 500 mL

of reagent 1 was placed in a water bath at 37 °C for 2 minutes, then 10 µL of the sample (homogenized intestine) was added and placed in a bain-marie bath at 37 °C for 7 minutes and 30 seconds. Subsequently 500 µL of reagent 2 plus 4.0 mL of distilled water were added, mixed and measured with absorbance at 660 nm. The blue color was proportional to the amylase activity in the sample.

To measure nonspecific alkaline proteolytic activity, a 1% casein solution was used as the substrate of the reaction. The incubation mixture was 250-400 mL of 0.1M Tris/ HCl buffer (pH 8.0). After incubation of the mixture for 30 minutes at 35 °C, the reaction was stopped by the addition of 1.0 mL of 15% TCA, then centrifuged at 1800 G for 10 minutes (Walter, 1984). 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 mg of tyrosine per minute.

Lipase activity was determined following the protocol of the Bioclin® commercial kit. Fifty µL of the sample was added to 1.0 mL of reagent 1 plus 50 µL of reagent 2 plus 100 µL of reagent 3; then the mix placed in a water bath at 37 °C for 2 minutes, after which 100 µL of reagent 4 was added and placed in a water bath at 37 °C for 30 minutes, then 2.0 mL of reagent 5 was added, centrifuged at 3,500 rpm for 3 minutes and measured with an absorbance wavelength of 410 nm.

The activity of the enzyme aspartate aminotransferase (AST) was determined using a commercial biochemical colorimetric test kit (Labtest®). Ten µL of sample were used, performing enzyme activity absorbance at a wavelength of 340 nm, and its reaction rate was measured at one- and three-minute intervals. The activity of the enzyme was expressed in moles per min (U) per mg of tissue.

Haematological parameters

Hematological parameters were determined using blood samples collected through a caudal vessel puncture using syringes containing 10% EDTA. After collection, the blood samples were placed in microtubes and refrigerated for further analysis. The hematocrit was determined by the microhematocrit technique, in a capillary tube with a length of 75 mm and an internal diameter of 1.0 mm and external diameter and 1.5 mm. In this tube, whole blood was inserted by pressure difference, then closed at one end and centrifuged at 12,000 rpm for 5 minutes.

Table 2. Mean values of growth performance of Nile tilapia juveniles fed the experimental diets containing increasing levels of inclusion of mesquite pod meal for 45 days.

Variable ¹	Levels of inclusion (%)					<i>p</i>	Regression*		CV(%) ²
	26	32	38	44	50		Linear	Quadratic	
FBW (g)	39.29	39.98	46.65	48.89	42.15	0.0005	0.0065	0.0067	16.68
FI (g)	19.12	18.07	20.88	21.80	19.75	0.0006	0.0064	0.0074	26.09
FCR	1.32	1.24	1.11	1.12	1.23	0.6033	-	-	10.59
SUR (%)	100	100	100	100	100	-	-	-	-

¹FBW: final body weight = $y = -0.041x^2 + 3.461x - 24.854$; $R^2 = 0.525$; FI: feed intake; FCR: feed conversion ratio; SUR: survival rate. ²CV: Coefficient of variation. *Significant regression model ($P < 0.05$).

The percentage of erythrocyte sedimentation was measured on a standardized scale of cell volume. For erythrocyte counts, 10 µL of whole blood was diluted in 2 mL of the solution (formaldehyde-citrate) and then homogenized. After 10 minutes of rest, the erythrocytes were counted in a Neubauer chamber, using an optical microscope with a magnification of 400 times. The erythrocytes were counted in five areas of 0.04 mm² and values were expressed in units per L of blood.

The concentration of hemoglobin was performed by the Collier method (1944), in which 2.0 mL of the Drabkin reagent were mixed with 10 µL of blood. The material was centrifuged, and the supernatant was used for the hemoglobin dosage in a spectrophotometer at 540 nm absorbance. The hemoglobin concentration was calculated using the formula: $Hb (g dL^{-1}) = \text{Sample Absorbance} \times \text{Correction Factor}$, expressed in $g dL^{-1}$. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the method recommended by Wintrobe (1934).

Statistical analysis

A completely randomized design with five treatments and four replicates was used. The data of growth performance, metabolic, enzymatic and hematological parameters were submitted to the Shapiro-Wilk test of normality of residues and analysis of variance (ANOVA). When the result was significant ($P < 0.05$), a regression analysis was performed with the statistical package *ExpDes.pt* in the R statistical software.

RESULTS

Performance

Significant differences in final body weight (FBW) and feed intake (FI) were observed between treatments ($P < 0.05$). There was no effect of MPM inclusion levels on FCR and SUR ($P > 0.05$), (Table 2).

Quadratic behavior with a positive effect was observed for the final mean weight of the animals with the maximum inclusion point of 38.67% of the MPM, (Figure 1).

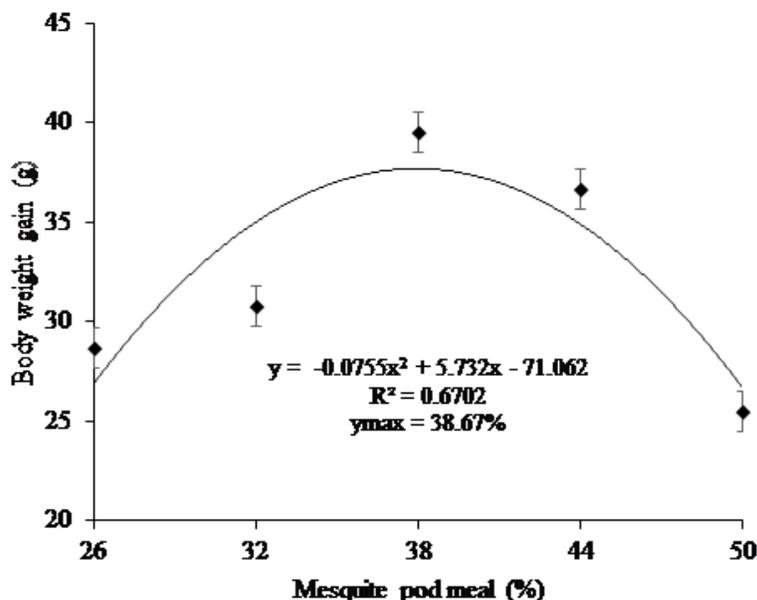


Figure 1. Final body weight of Nile tilapia juveniles fed diets containing graded levels of mesquite pod meal for 45 days.

Metabolites

The results indicated quadratic behavior for plasma glucose concentrations and energy reserve in the liver; the hepatic glycogen (P<0.05). The increase in MPM inclusion levels in the diets determined an increasing linear effect for the muscular energy reserve. There was no effect of the diets on the other metabolites (P>0.05), (Table 3).

Activity of digestive and transamination enzymes

There was no effect of the diets on the responses of the activities of the digestive enzymes (amylase, nonspecific alkaline protease

and lipase) and on the activity of the transamination enzyme (AST) evaluated in fish liver (P>0.05), (Table 4).

Haematological parameters

Hematological variables (hematocrit, hemoglobin, erythrocytes) and hematimetric indexes (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) in the fish did not show significant changes by MPM inclusion levels (P>0,05), (Table 5).

Table 3. Mean values of the metabolic indices analyzed in Nile tilapia fed experimental diets containing increasing levels of inclusion of the mesquite pod meal for 45 days.

Variable ¹	Levels of inclusion (%)					p	Regression*		CV(%) ²
	26	32	38	44	50		Linear	Quadratic	
Glc (mg dL ⁻¹)	65.32	66.26	73.82	67.46	67.16	0.0119	0.0199	0.0081	10.37
Gg H (mg of glucose, g of tissue ⁻¹)	5.36	16.17	20.21	17.44	9.00	2.944	0.1039	0	22.89
Gg M (mg of glucose, g of tissue ⁻¹)	0.62	0.80	0.67	0.99	1.04	0.0359	0.0091	0.8702	26.17
Prt (g dL ⁻¹)	3.33	3.38	3.77	3.26	3.39	0.8058	-	-	18.45
Fa (µmol mL ⁻¹)	28.35	27.37	29.95	30.31	26.92	0.9296	-	-	23.13
Alb (mg dL ⁻¹)	1.42	1.11	1.34	0.97	1.31	0.399	-	-	28.84
Trg (mg dL ⁻¹)	73.59	89.33	113.78	117.76	134.62	0.0972	-	-	29.62
Cls (mg dL ⁻¹)	77.26	77.19	87.16	91.08	95.54	0.2158	-	-	15.02

¹Glc: Glucose=y=-0.078x²+6.390x-58.477; R²=0.859; Gg H: Glycogen analyzed in the liver = y=-0.089x²+6.974x-115.118; R²=0.999; Gg M: Glycogen analyzed in muscle=y=-0.00013x²+0.007x-0.3625; R²=0.659; Prt: Total protein; Fa: Total free amino acids; Alb: Albumin, Trg: Triglycerides; Cls: Cholesterol. ²CV: Coefficient of variation *Significant regression model (P<0.05)

Table 4. Mean values for the enzymatic activities analyzed in Nile tilapia fed experimental diets containing levels of inclusion of the mesquite pod meal for 45 days.

	Levels of inclusion (%)					<i>p</i>	CV(%) ²
	26	32	38	44	50		
Digestive enzyme activity	65.32	66.26	73.82	67.46	67.16	0.0119	10.37
Intestinal Tissue	5.36	16.17	20.21	17.44	9.00	2.944	22.89
Amylase (U mg t ⁻¹) ¹	9.74	8.12	7.83	8.14	9.41	0.1667	14.31
Nonspecific alkaline protease (µg tyr min ⁻¹). g t ⁻¹ ²	4.58	5.57	4.93	4.46	5.17	0.6159	22.15
Lipase (U mg t ⁻¹) ¹	1.94	2.55	2.31	2.63	2.99	0.4515	31.84
Transamination enzyme activity							
Hepatic Tissue							
AST ³ (U mg ⁻¹). gt ⁻¹⁴	2.79	2.50	2.48	2.48	1.08	0.3134	52.37

¹(U mg t-1): Unit per milligram of tissue; ²(µg tyr min⁻¹). g t⁻¹: Microgram of tyrosine per minute per gram of tissue; ³AST: Aspartate aminotransferase; ⁴(U mg⁻¹). g t⁻¹: Unit per milligram per gram of tissue; ⁵CV: Coefficient of variation.

Table 5. Mean values for hematological variables analyzed in the blood plasma of Nile tilapia juveniles fed experimental diets containing increasing levels of inclusion of the mesquite pod meal for 45 days.

Variable ¹	Levels of inclusion (%)					<i>p</i>	CV(%) ²
	26	32	38	44	50		
Ht (%)	37.25	37.08	34.75	34.67	29.68	0.4413	22.12
Hb (g dL ⁻¹)	11.89	10.65	12.51	13.11	10.62	0.4916	29.26
RBC (x106.µL ⁻¹)	1.57	1.70	1.77	1.72	1.84	0.9482	36.38
MCV (fL)	251.31	235.69	219.86	229.98	191.30	0.7510	39.49
MCH	79.22	64.49	78.00	71.18	66.70	0.3103	15.92
MCHC (g dL ⁻¹)	32.54	29.98	36.88	39.15	41.38	0.5181	41.13

¹Ht: Hematocrit; Hb: Hemoglobin; RBC: Erythrocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration. ²CV: Coefficient of variation.

DISCUSSION

The performance of the fish can be influenced by the feed and its quantities present in the diets. It has been found that even certain levels of inclusion of alternative feeds provide better fish performance indices. In red tilapia (*Oreochromis mossambicus* X *Oreochromis niloticus*) up to 30% of sorghum starch can be included in the diets without compromising performance (Abdel and Atallah, 2016), according to Azevedo et al. (2017), the inclusion of up to 6.2% of flour of the bean byproduct as a substitute of soybean meal does not diminish the performance of tilapia. Other studies also did not record a reduction in the performance of Nile tilapia with the inclusion of other legumes as an alternative source in the diets. The inclusion of up 24% of *Vicia faba* fava beans (Azaza et al., 2009) did not result in a decrease in the performance of Nile tilapia juvenile. Even in tilapia larvae, it was possible to include up to 36.8% of *Vigna unguiculata* (Lara-Flores et al., 2007; Olivera-Castillo et al., 2011) without impairing performance.

The use of carbohydrates varies greatly among fish species, and the appropriate carbohydrate can improve fish growth and feed efficiency (Abdel and Atallah, 2016). The effects of alternative

sources of carbohydrates on growth parameters also depend on their percentage of inclusion (Signor et al., 2007). In the present study, an improvement in tilapia performance was observed with inclusion of up to 38,67% MPM in diets as an energy source. This fact can be explained by the better feed conversion ratio and the average fish feed intake. A decrease in fish growth performance, however, without any health consequences was observed in the studies of Jesus et al. (2011), Sena et al. (2012) and Silva et al. (2015) of Nile tilapia juveniles fed with MPM of above 20% inclusion in diets.

Silva et al. (2015), when replacing maize with mesquite meal in juvenile tilapia diets cultivated at low temperatures (20 °C), observed that the survival rate increased linearly with the inclusion levels of mesquite meal in the diet and that the presence of antinutritional factors present in small amounts in the diet would have stimulated the immune system of the fish. The presence of phytochemicals with antioxidant (Sirmah et al., 2011) and antimicrobial (Raja et al., 2012; Thakur et al., 2014) activities has already been confirmed in leaves, wood and bark of the mesquite plant. It is plausible then that the pods and seeds of the plant also contain substances with similar activities. Thus, the total survival of fish fed with mesquite meal would be related to a possible protective action of these substances.

Among the metabolic variables studied in fish nutrition, glycemia reflects on nutritional status and can be influenced by species, stages of life, diet, hormonal, physiological and behavioral regimes (Polakof et al., 2012; Kamalam et al., 2017). Glucose levels may indicate the carbohydrate source used and the percent of inclusion for fish species (Booth et al., 2013). In this study, the level of carbohydrates present in diets may have contributed to the increase in glycemia, but considered normal values for Nile tilapia (Tavares-Dias and Mariano, 2015). The mesquite pod meal is mostly composed of carbohydrates, a fact that presupposes the availability of carbon skeletons for glucose synthesis, causing a glycemic increase (Braga et al., 2010). When glucose is ingested above what is needed, it is converted to glycogen, which is stored in the liver and muscle, and its mobilization is controlled by the action of hormones and enzymes, or it is converted into lipids. This fact explains the occurrence of glycogenesis, by the availability of blood glucose by the carbohydrate source (MPM), leading to the synthesis of glycogen in the liver from glucose. The Nile tilapia can use high carbohydrate levels without any deregulation of glucose homeostasis (Boonanutanasarn et al., 2018).

The increase in muscle glycogen of tilapia ($P < 0.05$), caused by the inclusion of MPM in the diet, may be an indication for improvement in the meat quality, which has a greater availability of energy reserves for *rigor mortis*. The nutritional qualities of the muscle depend mainly on the nutritional value of the fats, carbohydrates and proteins that make up the feed (Listrat et al., 2016). The greater presence of muscle glycogen improves pH reduction during butchering, lactate production and consequently improves in meat quality (Gagaoua et al., 2015). Therefore, the inclusion of MPM in the diet promotes a metabolic profile of storage of carbohydrate reserves in the muscle.

Plasma total protein concentration is related to protein metabolism and nutritional conditions (Pohlenz and Gatlim, 2014), as well as the concentration of albumin, which is the main nutrient carrier protein that directly reflects the nutritional status of the animal and serves as a reference for evaluating the immune system of the fish (Yilmaz and Ergün, 2012). It was observed that the free plasma amino acid content had no effect on the transamination process, which, together with the mobilization of reserves, did not differ significantly with the increase in MPM levels. The reduction or increase in plasma concentration of total free amino acids is an adaptation to the needs for cell maintenance and its response is rapid in the absorption process, which was observed in *Oreochromis niloticus* studies fed with synthetic amino acids in their diet (Kamalam et al., 2017).

Likewise, with data from the present study, it was observed that the plasma concentration of total triglycerides and cholesterol were also not affected by MPM levels. These metabolites can be affected by feed and its composition and may depend on the proportions of the nutrients that are part of the diet. They represent a form of fat that circulates in the bloodstream and are stored in the

adipose tissue of the body, who in addition to the type of fat, excess carbohydrates can raise plasma triglycerides and total cholesterol levels (Marzzoco and Torres, 2010). In this case, the response obtained from these metabolites can be considered positive, since the MPM in the diets with different concentrations tested did not alter the values of triglycerides and total plasma cholesterol.

The presence of phenolic compounds has been reported as a cause of low performance in Nile tilapia (Pinto et al., 2000; Pinto et al., 2004). Mesquite seeds as feed may have their biological value reduced due to the presence of antinutritional factors, such as protease inhibitors, polyphenols, nitrates, oxalates, phytates, saponins and hemagglutinins (Damasceno et al., 2017). The tilapias present the capacity to adapt and adjust their digestive processes (Lin and Luo, 2011). This fact may explain the findings of the present study, in which the activity of the digestive enzymes (amylase, nonspecific alkaline protease and lipase) were not affected by MPM inclusion levels. This maintenance was given by the use of isoenergetic, isoproteic and isofibrous diets in the different treatments.

Secretion of the enzymes depends on the composition of the feed ingested daily, its frequency, amount of feed consumed, temperature and stage of development (Enes et al., 2010). Digestive enzyme activities and digestive transit times along the gastrointestinal tract may be affected by diet composition (Diógenes et al., 2018), which did not occur in this study.

Another response that favored the inclusion of MPM in diets was the enzymatic activity that is involved in amino acid metabolism (liver transamination), which did not alter its activity at the different inclusion levels. In tilapias, reductions of aspartate aminotransferase (AST) activity were observed in plant-based diets and it was suggested that the reduction occurs by distancing the nitrogen products from the oxidative pathways (Deng et al., 2017). In addition to plant sources, Xiong et al. (2014) observed that high protein/ starch ratios increased AST activities. These studies indicate that nutrients and feed sources are capable of producing transamination in fish, this was not observed in the present study.

The MPM inclusion is efficient as an alternative feed in diets for tilapia juveniles, since no differences were also observed in the blood variables. Thus, the feed tested did not compromise the health status of the animals, since the values observed complied with the values described for the species (Tavares-Dias and Mariano, 2015).

CONCLUSION

Mesquite pod meal may incorporate up to 38.67% in the diet of juvenile Nile tilapia, with positive effects on growth performance, metabolism, digestive enzyme activities and transamination, as well as not compromising the health of the species under study. What indicates mesquite flour may be an alternative food in the formulation of diets for juveniles of Nile tilapia.

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