Tropical Animal Health and Production (2021) 53:410
https://doi.org/10.1007/s11250-021-02826-9

REGULAR ARTICLES

# Corn substitution by mesquite bean flour (*Prosopis juliflora*) maintains growth and improves protein metabolism of Nile tilapia juveniles (*Oreochromis niloticus*)

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Received: 27 December 2020 / Accepted: 18 June 2021


## Abstract

The objective of this study was to evaluate the potential of mesquite bean flour (Prosopis juliflora) as an energy ingredient in extruded diets for juvenile Nile tilapia (Oreochromis niloticus). Two experiments were carried out: the first consisted of a study to evaluate the chemical composition and digestibility of the energy and nutrients of MBF; the second consisted of a growth test, in which juveniles consumed diets containing different proportions of corn substitution by MBF, in which zootechnical, hematological, physiological, and metabolic variables were evaluated. It was observed that MBF has a chemical composition similar to corn, as well as the apparent digestibility of energy and nutrients. The higher sucrose/starch ratio of the ingredient stands out, as well as the difference in digestibility ($p < 0.05$) between the predominant carbohydrates 87.63 and 99.25% for starch and sucrose, respectively. In the growth assay, no difference was observed between zootechnical variables ($p > 0.05$), and sucrase and alkaline phosphatase activities were increased ($p < 0.05$), which was not observed for amylase and lipase ($p > 0.05$). The hematological variables did not change ($p > 0.05$). Metabolic variables indicate a reduction in gluconeogenesis from amino acids, as can be seen by the reduction in liver transaminase levels (ALT and AST) and glutamate dehydrogenase (GDH), as well as the greater availability of free amino acids in plasmas ($p < 0.05$). Thus, it can be said that MBF has a high nutritional value and can totally replace corn in diets for juvenile tilapia and the metabolic findings indicate a potential protein-sparing effect.

**Keywords** Semiarid · Alternative ingredient · Carbohydrate · Sucrose · Protein sparing

## Introduction

Nile tilapia is one of the most important species in aquaculture worldwide, due to its adaptation to captivity, satisfactory growth, and good quality flesh, with its production concentrated in tropical and subtropical regions (El-Sayed 2006). In the last decades the use of intensified aquaculture systems has caused a great increase in their production (Suresh and Bhujel 2019), increasing the pressure on the quality of the diets, mainly regarding the discharge of effluents and fish health (NRC 2011). The species has an omnivorous eating habit which makes it adapted to a wide range of ingredients used by the feed industry, highlighting those rich in carbohydrates (Schrama, 2018; Wilson 1994).

Carbohydrates are used in animal nutrition due to their energy concentration and low cost, and they also play a role in the physical characteristics of processed diets (Hertrampf and Piedad-Pascual 2000). Starch is a polysaccharide formed

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Published online: 23 July 2021

by glucose units, and it is the main form of energy storage in cereals, among them corn and wheat, the most used energy ingredients in diets for Nile tilapia (Stone 2003). Thus, starch is an important source of energy in Nile tilapia diets (NRC 2011). On the other hand, alternative ingredients, such as mesquite, have their energy reserves in the form of simpler carbohydrates such as sucrose (Sawal et al. 2004), a disaccharide formed by glucose and fructose (Stone 2003).

While starch needs hundreds of hydrolysis reactions for its monosaccharides to be released and absorbed, only one process of hydrolysis releases the monosaccharides from sucrose for absorption (Hemre and Deng 2015). In addition, fructose metabolism produces more Acetyl-CoA when compared with glucose, consequently generating more energy in the Krebs cycle (Dabrowski and Guderley 2003; Hemre and Deng 2015). These physiological and metabolic changes can cause changes in the metabolism of other nutrients, including the oxidation of amino acids (Corrêia, 2019; Cui, 2010, Xu et al. 2020).

The mesquite tree (*Prosopis juliflora*) is an arboreal legume native to the Mexican semiarid to some Andean countries in South America, introduced and currently considered invasive in other semiarid regions of the world (Harris, 2003). Naturally adapted to water scarcity, its production is concentrated in the driest periods of the year, precisely the off-season of conventional grains (Sawal et al. 2004). Its pods are rich in soluble carbohydrates, mainly sucrose (Sawal et al. 2004), and its proximal composition (88.0 g $kg^{-1}$ of crude protein, 4.501 kcal $kg^{-1}$ gross energy) is similar to that of corn (82.0 g $kg^{-1}$ of crude protein and 4.216 kcal $kg^{-1}$ of gross energy) (Rostagno 2011; Sawal et al. 2004). The processing of mesquite can give rise to different ingredients for animal feed, including the seed, separated from the husk and later ground, considered a protein ingredient; mesquite bean meal (MBM), obtained by drying and grinding the whole pod, considered an energetic ingredient; and mesquite bean flour (MBF), obtained from the peel after grinding by forced ventilation, considered an energetic ingredient (Bhatt et al. 2011; Sawal et al. 2004).

Grain processing promotes a series of changes in the structure of ingredients. It can increase the availability of nutrients, make them more palatable, decrease fiber, and consequently reduce anti-nutritional factors (Correia, 2008). Corn, wheat, and respective co-products have different digestibility coefficients for dry matter (DM), gross energy (GE), crude protein (CP), and amino acids (AAs) and a strong correlation with the nutritional value and chemical composition of the ingredients (Vidal, 2015; Vidal, 2017).

De Souza, (2019) evaluated the digestibility and potential for inclusion of MBM in extruded diets for Nile tilapia. In general, the authors observed high use of energy and nutrients, highlighting the greater digestibility of sucrose (88.99%), compared with starch (72.19%). On the other hand, the presence of anti-nutritional factors limited the substitution of dietary corn to 20%, corresponding to 6% of inclusion of MBM. Nevertheless, no deleterious effects to the health of the fish were observed that demonstrated good adaptive capacity, as observed by the activity of the enzymes amylase and sucrase.

This study aimed to evaluate the potential use of MBF in extruded diets for Nile tilapia, considering the nutritional value, performance, and physiological and metabolic responses.

## Material and methods

Experiments were conducted in two stages at the Laboratório de Aquicultura da Universidade Federal do Vale do São Francisco (UNIVASF), Campus de Ciências Agrárias, in Petrolina, Pernambuco, Brazil. Nile tilapia juveniles were obtained from the 3ª Superintendencia da Companhia de Desenvolvimento do Vale do São Francisco e Parnaíba, in Petrolina, Pernambuco, Brazil.

During the experiments, water temperature and dissolved oxygen were measured every day, in the morning and afternoon. Ammonia and pH were measured daily, in the morning. pH and a were measured in the morning. The measurements were performed using a multiparameter analyzer (Hanna, HI9829). In this way, the parameters were kept within the ideal conditions for the species (Caldini, 2011): temperature, $27.12 \pm 0.10$ °C; dissolved oxygen, $6.64 \pm 0.12$ mg $L^{-1}$; unionized ammonia, maintained below 0.03 mg $L^{-1}$; and pH, $7. \pm 0.03$.

### Digestibility trial

#### Diet preparation

The reference diet was formulated from practical ingredients (Table 1), to meet the energy and digestible nutrient requirements of Nile tilapia juveniles (Furuya et al. 2010). The test diet (Table 2) consists of 70% of the reference diet and 30% of the MBF (NRC 2011).

MBF was supplied by Riocon®, a company located in the municipality of Manoel Vitorino, Bahia, Brazil. The processing of mesquite pods followed the following flowchart at the company: drying at temperatures ranging from 60 to 80 °C, followed by milling in an industrial hammer mill, finished with the separation of the shells by forced ventilation process (Table 2).

The manufacture of the reference and test diets was carried out according to the following sequence: grinding, weighing, and homogenizing, adding water (12% of the total weight). The mixture was extruded in an small extruder (Ex-Micro, Exteec, Ribeirão Preto, São Paulo, Brazil), at 100 °C and 44 psi, using a 2-mm die, and then dehydrated in a forced-ventilation oven at 55 °C for 24 h.

**Table 1** Formulation of the reference diet of the in vivo digestibility trial of mesquite bean flour

| Ingredient | g $kg^{-1}$ |
|---|---|
| Poultry viscera flour | 305.0 |
| Wheat bran | 263.8 |
| Corn meal | 180.9 |
| Corn gluten 60% | 130.0 |
| Soybean meal 45% | 106.0 |
| Mineral and vitamin supplement[1] | 5.0 |
| Dicalcium phosphate | 5.0 |
| Vitamin C[2] | 2.0 |
| Antifungal[3] | 1.0 |
| Chromium oxide | 1.0 |
| Antioxidant[4] | 0.2 |

[1]Mineral and vitamin supplement (per kg): vitamin A, 1,200,000 IU; vitamin D3, 200,000 IU; vitamin E, 12,000 mg; vitamin K3, 2400 mg; vitamin B1, 4800 mg; vitamin B2, 4800 mg; vitamin; B6, 4000 mg; vitamin B12, 4800 mg; folic acid, 1200 mg; D-calcium pantothenate, 12,000 mg; ascorbic acid, 48,000 mg; biotin, 48 mg; choline, 65,000 mg; nicotinic acid, 24,000 mg; iron, 10,000 mg; copper sulfate, 600 mg; manganese sulfate, 4000 mg; zinc sulfate, 6000 mg; potassium iodine, 20 mg; cobalt, 2 mg; selenium, 20 mg; 2Vitamin C: calcitic salt, active principle-42% ascorbic acid2-monophosphate / [2]Vitamin C resistant to high pressures and temperatures / [3]Calcium propionate / [4]Butyl-hydroxy-toluene

**Table 2** Chemical composition and amino acid profile of the reference diet and mesquite bean flour

| Nutrient (g $kg^{-1}$) | Item | |
|---|---|---|
| | RD | MBF |
| Crude protein | 400.4 | 97.40 |
| Gross energy (kcal $kg^{-1}$) | 4626 | 4646 |
| Crude fiber | 40.1 | 69.6 |
| Ethereal extract | 63.5 | 30.54 |
| Starch | * | 142.4 |
| Sucrose | * | 525.9 |
| Total phenols | * | 0.48 |
| Tannins (mg $kg^{-1}$) | * | 39.15 |
| *Essential amino acids* | | |
| Arginine | 22.9 | 1.82 |
| Phenylalanine | 21.2 | 1.61 |
| Histidine | 7.0 | 0.53 |
| Isoleucine | 18.0 | 1.38 |
| Leucine | 42.5 | 3.27 |
| Lysine | 18.9 | 1.46 |
| Methionine | 7.2 | 0.46 |
| Threonine | 16.3 | 1.33 |
| Tryptophan | 2.8 | 0.22 |
| Valine | 20.7 | 1.72 |
| *Non-essential amino acids* | | |
| Aspartic acid | 33.7 | 2.74 |
| Glutamic acid | 71.5 | 5.56 |
| Alanine | 33.6 | 2.58 |
| Cystine | 5.9 | 0.41 |
| Glycine | 27.3 | 2.17 |
| Proline | 31.8 | 3.02 |
| Serine | 22.7 | 1.75 |
| Tyrosine | 13.5 | 1.07 |

Crude protein: N×6.25 / RD: reference Diet; MBF: mesquite bean flour / *: Not measured

### Study animals and experimental design

For the digestibility and growth experiments, two batches of sexually reversed male tilapia were used. In both experiments, the animals were acclimated through a period of 10 days to adapt to the laboratory, in which they were housed in 2000-L tanks with a continuous water flow system and fed twice a day with a commercial diet until apparent satiety. Thus, two adaptation periods were carried out, for the digestibility experiment and for the growth experiment, performed subsequently.

After the adaptation period, the 160 fish from the digestibility experiment (average weight 25.88 ± 5.52 g) were placed in eight conical tanks with a useful volume of 250 L. Thus, feces corresponding to the reference and test diets were collected in quadruplicate, with each tank containing 20 fish representing an experimental unit. The fish were acclimated to the diets (reference and test) and to the management routine for 7 days, and after that period the feces were collected for 10 days, to obtain the quantity of fecal material necessary for laboratory analysis.

Feces were collected using an adapted Guelph system (Pezzato, 2002). The fish were fed until apparent satiety, every day between 11:00 and 16:00. Thus, the excretion of feces was concentrated between late afternoon and evening, synchronizing with collection time and optimizing the experimental period. At 16:30 the tanks were cleaned and the water changed at 17:00, thus preventing feed pellets from being regurgitated during collection and consequently contamination of feces (De Souza, Silva, Felix e Silva, Campeche, Melo and Vidal 2019, Vidal, Xavier, Michelato, Martins, Pezzato and Furuya 2015). In the morning (08:00), the feces that sedimented during the night was collected, separated from excess water, packaged, properly identified, and kept in a freezer − 21 °C until the end of the collection period. (NRC 2011).

### Chemical analysis of ingredients, diets, and feces

All samples were dried in a forced ventilation oven for 48 h and ground in a hammer mill. The dry matter was determined by gravimetric methods, the crude protein was determined by the Micro-Kjeldahl method, and ether extract was extracted

by the Soxhlet method and the ashes obtained by burning the organic matter in a muffle; all analyses are described by AOAC (2005). The gross energy of the samples was determined by burning in an adiabatic calorimetric bomb (Parr 1266, Parr Instruments Co., Moline, Illinois, USA). The amino acids were determined by the commercial laboratory CBO, located in the municipality of Campinas, São Paulo, Brazil. Chromium (III) oxide ($Cr_2O_3$) was determined according to the methodology described by Bremer Neto, (2003). The procedures for measuring sucrose and starch content followed the methods described by Boyes (1958) and Makkar (2003), respectively. Total phenols and tannins in MBF and in experimental diets were determined according to the AOAC (2005).

### Apparent digestibility coefficients (ADC) calculation

The apparent digestibility coefficients (ADC) of the energy and nutrients in the diets were calculated according to the concentration of chromium oxide added to the diets and which was recovered in the feces. The calculations were performed according to the equation described below (NRC 2011):

$$ADC = 100 - \left[100 \times \left(\frac{gkg^{-1}I_D}{gkg^{-1}I_F}\right) \times \left(\frac{gkg^{-1}N_F}{gkg^{-1}N_D}\right)\right]$$

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

$$ADC_{ing} = ADC_{ED} + \left(ADC_{ED} - ADC_{RD}\right) \times \left[\left(\frac{b \times N_{RD}}{a \times N_{ing}}\right)\right]$$

where $ADC_{ing}$ = apparent digestibility coefficient of the ingredients, $ADC_{ED}$ = apparent digestibility coefficient of the experimental diet, $ADC_{RD}$ = apparent digestibility coefficient of the reference diet, a = experimental ingredient percentage, b = reference diet percentage, $N_{RD}$ = nutrients in the reference diet, and $N_{ing}$ = nutrients in the experimental diet (NRC 2011).

## Substitution of corn by MBF

### Experimental diets

Six practical diets were formulated with different levels of substitution of corn for MBF, 0, 20, 40, 60, 80, and 100% (Table 3), corresponding to treatments D0, D20, D40, D60, D80, and D100. The diets were balanced based on energy and digestible protein, digestible lysine, digestible methionine, and available phosphorus, in order to meet the nutritional requirements of Nile tilapia juveniles (Furuya, Pezzato, Barros, Boscolo, Cyrino, Furuya and Feiden 2010). All diet processing procedures followed the same protocol established for the digestibility experiment.

### Study animals and experimental conditions

As in the digestibility experiment, sexually reversed male Nile tilapia juveniles were used. The management and facilities used to adapt the fish to the laboratory were also similar to those described in the digestibility experiment. After the adaptation period, 384 fish (initial average weight of 21.85 ± 1.01 g) were divided into 24 plastic tanks with individual capacity of 1000 L, in a water recirculation system, coupled to a radial blower, to maintain oxygenation from water. Under these conditions, the fish were fed until apparent satiety four times a day, between 8:00 and 18:00, for 45 days. The design of the experiment was completely randomized, with six treatments and four replications, with each plastic tank representing an experimental unit.

Fish performance was assessed according to the following zootechnical parameters:

Final weight (FBW, g).

Final biomass (FB, g) = sum of the weights of all animals per experimental unit.

Weight gain (WG, g) = (final weight − initial weight).

Biomass gain (BG, g) = (final biomass − initial biomass)

$$\text{Feed intake(FI, g)} = \frac{\text{Total of consumed ration (g)}}{\text{Number of fish per repetition}}$$

$$* \text{ Thermal coefficient of growth (TCG)} = \left[\left(\sqrt[3]{Fw} - \sqrt[3]{Iw}\right)/(T \times t)\right] \times 1000$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Survival (S, \%)} = \frac{\text{(final number of fish)}}{\text{(initial number of fish)}} \times 100$$

$$\text{Carcass yield (CY, \%)} = [\text{(live weight)} - \text{carcass weight (eviscerated)}] \times 100$$

*Fw = final weight, Iw = initial weight, T = average temperature, t = days.

### Metabolic analysis

At the end of the experiment, blood, liver, muscle, and intestine samples were collected from 12 fish, from each experimental unit. The fish were anesthetized with benzocaine (1 g 10 L-1) and the blood collected in heparinized

**Table 3** Formulation and proximal composition of the experimental diets for Nile tilapia with corn substitution by mesquite bean flour

| Ingredient | Corn substitution by MBF (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 |
| Corn | 300.0 | 240.0 | 180.0 | 120.0 | 60.0 | 0.0 |
| Mesquite bean flour | 0.0 | 60.0 | 120.0 | 180.0 | 240.0 | 300.0 |
| Wheat middlings | 251.8 | 254.9 | 258.1 | 261.2 | 264.4 | 267.5 |
| Poultry by-product meal | 160.0 | 160.0 | 160.0 | 160.0 | 160.0 | 160.0 |
| Soybean meal | 149.0 | 149.0 | 149.0 | 149.0 | 149.0 | 149.0 |
| Corn gluten meal | 101.0 | 101.0 | 101.0 | 101.0 | 101.0 | 101.0 |
| Microcrystalline cellulose | 14.0 | 11.2 | 8.4 | 5.6 | 2.8 | 0.0 |
| Dl-methionine | 5.0 | 4.6 | 4.2 | 3.7 | 3.3 | 2.9 |
| Mineral and vitamin supplement [2] | 6.0 | 6.1 | 6.2 | 6.2 | 6.3 | 6.4 |
| Dicalcium phosphate | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| L-lysine HCl | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin C[3] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Antifungal[4] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Antioxidant[5] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| *Nutrients (g. kg$^{-1}$)* | *Proximal composition calculated[6] and analyzed[7]* | | | | | |
| Digestible energy (kcal. kg-$^{1}$)[6] | 3195 | 3191 | 3208 | 3224 | 3261 | 3298 |
| Digestible protein[6] | 260.0 | 260.0 | 260.0 | 260.0 | 260.0 | 260.0 |
| Digestible arginine[6] | 14.2 | 14.3 | 14.4 | 14.4 | 14.5 | 14.6 |
| Digestible histidine[6] | 5.4 | 5.3 | 5.2 | 5.1 | 5.0 | 5.0 |
| Digestible isoleucine[6] | 10.3 | 10.3 | 10.3 | 10.4 | 10.4 | 10.4 |
| Digestible leucine[6] | 24.9 | 24.8 | 24.7 | 24.6 | 24.4 | 24.3 |
| Digestible lysine[6] | 17.6 | 17.8 | 18.0 | 18.1 | 18.3 | 18.5 |
| Digestible methionine[6] | 8.5 | 8.4 | 8.3 | 8.2 | 8.1 | 8.0 |
| Digestible phenylalanine[6] | 12.8 | 12.7 | 12.6 | 12.6 | 12.5 | 12.4 |
| Digestible threonine[6] | 7.1 | 7.2 | 7.4 | 7.5 | 7.7 | 7.8 |
| Digestible tryptophan[6] | 1.9 | 1.9 | 2.0 | 2.0 | 2.0 | 2.1 |
| Digestible valine[6] | 10.9 | 11.0 | 11.1 | 11.2 | 11.4 | 11.5 |
| Ether extract[7] | 31.9 | 29.5 | 24.8 | 23.9 | 22.7 | 21.8 |
| Crude fiber[7] | 48.5 | 47.4 | 44.6 | 45.4 | 46.4 | 44.3 |
| Total phenols[7] | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |
| Tannins (mg. kg$^{-1}$)[7] | 4.0 | 8.2 | 13.9 | 18.3 | 19.5 | 19.9 |
| Starch[7] | 62.9 | 58.8 | 54.6 | 51.0 | 46.3 | 42.2 |
| Sucrose[7] | 5.5 | 9.3 | 13.2 | 17.0 | 20.8 | 24.7 |
| Starch:Sucrose | 11.5 | 6.3 | 4.2 | 3.0 | 2.2 | 1.7 |

MBF: mesquite bean flour / [2] Mineral and vitamin mixture for fish- chemical composition: 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) 1.200.00 mg kg$^{-1}$; Nicotinic Acid (Minimum) 20.00 g kg$^{-1}$; Pantothenic 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$^{-1}$; / [3] Vitamin C resistant to high pressures and temperatures / [4]Calcium propionate/ [5]Butyl-hydroxy-toluene/ [6]According to Furuya, Pezzato, Barros, Boscolo, Cyrino, Furuya and Feiden (2010)

syringes, by puncture of the caudal vein, then euthanized by spinal cord section, and then the necessary tissues were removed.

Blood glucose analysis was performed using an electronic glucose meter (Accu-Chek®). Plasma was centrifuged at $1.800 \times g$ for 5 min. Samples were stored at − 20 °C. Total cholesterol (mg dL$^{-1}$), total proteins,

and triglycerides were assessed in plasma samples using the respective colorimetric tests (Labtest®). The content of total free amino acids was evaluated according to Bidinotto et al. (1997). Muscle, whole intestine, and liver were collected to assess triglycerides, total proteins (Labtest®), and hepatic glycogen (nmol $g^{-1}$) (according to (Bidinotto et al. 1997).

Muscle tissue, liver, and enzyme activity were determined by homogenization of the tissues in a buffer (10 mM phosphate/20 mM Tris-pH 7.0) for 10 min (4 °C) using a homogenizer (Marconi). Supernatant collection was performed to be used in the enzyme analysis. Intestinal amylase and lipase activities were assessed by using commercial kits (Amylases and Lipase BioClin®). Determination of alkaline proteolytic activity was performed using a 1% casein solution as substrate for the reaction. For incubation, a mixture composed of 250–400 µL of 1% azocasein and 0.1 M Tris/HCl buffer (pH 8.0) was used, incubating the mixture for 30 min at 35 °C. After this process, 1.0 mL of 15% trichloroacetic acid was added to stop the rection, followed by centrifugation at $1.800 \times g$ for 10 min (Walter 1981). Standard used was Tyrosine, and the unit used for enzymatic activity was set as the amount of enzyme needed to catalyze the formation of 1 µg of tyrosine per min.

For hepatic and muscular aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymatic activity, ALT and AST Liquiform® (Labtest®) commercial kits were used by means of the buffered extracts. It was used a semiautomatic biochemical analyzer (model 2000 UV) to perform the reading of the samples at a wavelength of 340 nm. Hepatic glutamate dehydrogenase (GDH) enzymatic activity was performed according to Hochachka and Hulbert (1978). The assessment was recorded for 1 min, with 15-s intervals. The medium of the reaction was composed of imidazole buffer pH 7.0 (0.05 M), 0.1-mM NADH, 1-mM ADP, 5-mM α-ketoglutarate, 250-mM ammonium acetate, and an adequate aliquot of the homogenate. To calculate enzymatic activity, a pre-determined NADH molar extinction coefficient ($\varepsilon 340 = 0.0855108$ $mmol^{-1}$ $L^{-1}$) was used. The result of enzyme activity was expressed in mole per min (U) per mg of protein (U $mg^{-1}$ protein).

### Hematological analyses

Regarding the determination of hematological parameters, whole blood samples were used. For total red blood cells, Neubauer's chamber was used to count cells. Hemoglobin concentration was evaluated using Drabkin's reagent followed by reading in a spectrophotometer at 540-nm absorbance. For the determination of hematocrit, the microhematocrit method (Ranzani-Paiva et al. 2013) was used. Data collected was used to calculate hematemesis indexes according to Wintrobe (1934).

$$\text{Mean corpuscular volume (MCV, fL)} = \frac{\text{Hematocrit} \times 10}{\text{Eritrocytes Number } (\times 10^6 \mu L^{-1})}$$

$$\text{Mean corpuscular hemoglobin (MCH, pg)} = \frac{\text{Hemoglobin Rate} \times 10}{\text{Eritrocytes Number}}$$

$$\text{Mean corpuscular hemoglobin concentration (MCHC, g dL}^{-1}\text{)} = \frac{\text{Hemoglobin Rate} \times 100}{\text{Hematocrit}}$$

### Statistical analysis

Data were tested for normality and homogeneity. For testing digestibility of soluble carbohydrates a *t* test was used. An analysis of variance (ANOVA) was performed to the

**Table 4** Apparent digestibility coefficient of the mesquite bean flour ($n = 4$)

| Nutrient (%) | Ingredient | |
|---|---|---|
| | MBF | SEM |
| Dry matter | 80.78 | 0.77 |
| Crude protein | 82.01 | 0.19 |
| Gross energy | 81.38 | 0.62 |
| Starch | 87.63[b] | 0.24 |
| Sucrose | 99.25[a] | 0.26 |
| *Essential amino acids* | | |
| Arginine | 83.92 | 0.19 |
| Phenylalanine | 84.55 | 0.21 |
| Histidine | 85.98 | 0.07 |
| Isoleucine | 84.96 | 0.16 |
| Leucine | 84.93 | 0.19 |
| Lysine | 82.88 | 0.17 |
| Methionine | 82.27 | 0.31 |
| Threonine | 82.25 | 0.09 |
| Tryptophan | 95.05 | 0.03 |
| Valine | 85.90 | 0.14 |
| *Non-essential amino acids* | | |
| Aspartic acid | 83.92 | 0.11 |
| Glutamic acid | 87.40 | 0.10 |
| Alanine | 82.79 | 0.19 |
| Cystine | 70.03 | 0.14 |
| Glycine | 79.93 | 0.34 |
| Proline | 84.87 | 0.17 |
| Serine | 82.44 | 0.19 |
| Tyrosine | 84.36 | 0.21 |

MBF: Mesquite bean flour / SEM: standard error of mean

Averages in the same column, followed by the same letter, do not differ by the *t* test ($p < 0.05$)

**Table 5** Growth performance Nile tilapia fed diets with corn substitution by mesquite bean flour ($n=4$)

| Parameter | Corn substitution by MBF (%) | | | | | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | | |
| Final body weight (g) | 56.59[a] | 62.95[a] | 57.19[a] | 60.70[a] | 55.75[a] | 57.54[a] | 0.92 | 0.1649 |
| Final biomass (g) | 496.58[a] | 573.85[a] | 505.45[a] | 546.48[a] | 486.28[a] | 504.45[a] | 11.08 | 0.9858 |
| Weight gain (g) | 41.38[a] | 47.82[a] | 42.12[a] | 45.54[a] | 40.52[a] | 42.04[a] | 0.92 | 0.1558 |
| Biomass gain (g) | 314.10[a] | 392.3[a] | 324.59[a] | 364.60[a] | 303.54[a] | 318.40[a] | 11.23 | 0.8558 |
| Feed intake (g) | 57.55[a] | 65.43[a] | 58.57[a] | 61.47[a] | 59.61[a] | 61.24[a] | 0.56 | 0.0503 |
| Feed conversion ratio | 1.40[a] | 1.37[a] | 1.40[a] | 1.35[a] | 1.48[a] | 1.49[a] | 0.02 | 0.5256 |
| Thermal growth coefficient | 1.14[a] | 1.26[a] | 1.16[a] | 1.22[a] | 1.12[a] | 1.14[a] | 0.02 | 0.1407 |
| Carcass yield (%) | 80.36[a] | 81.01[a] | 84.50[a] | 80.45[a] | 80.37[a] | 81.87[a] | 0.73 | 0.5553 |

MBF: Mesquite bean flour / SEM: standard error mean / Different letters on the same line are significantly different by Tukey's test ($p<0.05$)

other variables, and in sequence it was used Tukey's test for multiple comparison of the means.

Performance data were evaluated by means of polynomial regression and a linear response plateau analysis, following the model $Y = L + U \times (RX)$, where Y = value of the variable studied, X = percentage of corn in substitution of MBF, L = plateau response of the studied variable, U = slope of the line, and R = percentage of corn substitution by MBF estimated by the intercept point (Robbins et al. 1979). The model more suitable for each variable was chosen. Statistical software SAS University Edition (SAS 2017) was used for data analysis of all procedures.

## Results

### Digestibility of MBF

In general, the ADCs for energy and nutrients were high, with values above 80% (Table 4). The digestibility of soluble carbohydrates was high, with greater digestibility of sucrose compared with starch ($p<0.05$).

### Corn substitution by MBF

#### Zootechnical variables

The zootechnical indexes evaluated were not altered by the replacement of corn by MBF ($p>0.05$). During the experimental period, no mortality was observed among animals fed with treatment diets (Table 5).

#### Digestive enzyme activity

No difference was observed in the activity of the lipase and amylase enzymes. On the other hand, alkaline protease and sucrase varied according to the treatments ($p<0.05$) (Table 6).

It was observed that the activity of the alkaline protease (Fig. 1) increased until reaching a plateau at 39.54% of replacement of corn by MBF. On the other hand, sucrase activity (Fig. 2) remained on a plateau until 41.37% of corn substitution by MBF, from which point an increase in enzyme activity was observed.

#### Metabolism

The metabolic profile in the plasma, liver, muscle, and intestinal tissues of animals was modified by replacement of the corn by MBF (Table 7). Glycemic control of the animals

**Table 6** Digestive enzymatic activity in Nile tilapia fed diets with corn substitution by mesquite bean flour ($n=6$)

| Enzyme[1] | Corn substitution by MBF (%) | | | | | | SEM | Effect | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | | | |
| Amylase | 3.05[a] | 2.83[a] | 2.91[a] | 2.11[a] | 1.94[a] | 2.22[a] | 0.14 | - | 0.0565 |
| Alkaline protease | 2.27[c] | 3.54[bc] | 5.88[a] | 5.60[ab] | 5.28[ab] | 6.11[a] | 0.32 | LRP | <.0001 |
| Sucrase | 0.21[c] | 0.22[c] | 0.27[bc] | 0.33[abc] | 0.41[ab] | 0.52[a] | 0.03 | LRP | 0.0002 |
| Lipase | 8.74[a] | 7.45[a] | 8.37[a] | 9.11[a] | 7.07[a] | 8.96[a] | 0.23 | - | 0.0272 |

[1]Activity unit per milligram of protein / MBF: mesquite bean flour / SEM: Standard error of mean / Different letters on the same line are significantly different by Tukey's test ($p<0.05$)

**Table 7** Metabolic parameters of Nile tilapia fed diets with corn substitution by mesquite bean flour ($n=8$)

| Parameter | Corn substitution by MBF (%) | | | | | | SEM[2] | Effect | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | | | |
| *Plasma* | | | | | | | | | |
| Protein (g $dL^{-1}$) | $2.23^{c}$ | $3.19^{ab}$ | $2.87^{abc}$ | $2.59^{bc}$ | $3.33^{a}$ | $3.03^{ab}$ | 0.08 | - | 0.0002 |
| Triglycerides[4] (mg $dL^{-1}$) | $167.34^{a}$ | $168.36^{a}$ | $171.56^{a}$ | $151.88^{a}$ | $142.34^{ab}$ | $109.45^{b}$ | 4.81 | Linear | 0.0002 |
| Glucose (mg $dL^{-1}$) | $112.73^{c}$ | $142.62^{bc}$ | $143.99^{bc}$ | $220.56^{a}$ | $160.05^{b}$ | $124.35^{c}$ | 5.93 | - | < .0001 |
| Cholesterol (mg $dL^{-1}$) | $115.47^{a}$ | $108.64^{a}$ | $111.49^{a}$ | $96.51^{a}$ | $115.57^{a}$ | $110.36^{a}$ | 2.08 | - | 0.0818 |
| Total plasma amino acid (µmoles $ml^{-1}$) | $45.26^{cd}$ | $41.09^{d}$ | $59.76^{bcd}$ | $63.08^{bc}$ | $77.16^{b}$ | $106.86^{a}$ | 3.74 | LRP | < .0001 |
| *Liver* | | | | | | | | | |
| Protein (g $g^{-1}$) | $21.20^{a}$ | $22.32^{a}$ | $18.31^{a}$ | $15.68^{a}$ | $15.25^{a}$ | $16.17^{a}$ | 0.90 | - | 0.0781 |
| Triglycerides (mg $g^{-1}$) | $132.43^{a}$ | $130.27^{a}$ | $131.57^{a}$ | $122.43^{a}$ | $122.70^{a}$ | $118.34^{a}$ | 2.66 | - | 0.5603 |
| Glycogen (µmoles glucose $g^{-1}$ tissue) | $40.30^{a}$ | $30.71^{b}$ | $35.85^{ab}$ | $29.86^{b}$ | $20.67^{c}$ | $5.56^{d}$ | 1.82 | LRP | < .0001 |
| *Intestine* | | | | | | | | | |
| Protein (g $g^{-1}$) | $35.67^{a}$ | $37.64^{a}$ | $35.76^{a}$ | $33.06^{a}$ | $46.05^{a}$ | $33.49^{a}$ | 1.67 | - | 0.2400 |
| Triglycerides (mg $g^{-1}$) | $228.80^{a}$ | $214.25^{a}$ | $218.87^{a}$ | $222.54^{a}$ | $235.26^{a}$ | $233.59^{a}$ | 3.90 | - | 0.6046 |
| *Muscle* | | | | | | | | | |
| Protein (g $g^{-1}$) | $85.13^{a}$ | $77.47^{a}$ | $76.06^{a}$ | $86.07^{a}$ | $81.78^{a}$ | $84.21^{a}$ | 1.47 | - | 0.2471 |
| Triglycerides (mg $g^{-1}$) | $110.88^{a}$ | $103.01^{a}$ | $109.91^{a}$ | $109.01^{a}$ | $109.44^{a}$ | $98.88^{a}$ | 1.85 | - | 0.3641 |
| Glycogen[5] (µmoles glucose $g^{-1}$ tissue) | $23.31^{c}$ | $26.52^{bc}$ | $29.82^{abc}$ | $30.23^{ab}$ | $27.88^{bc}$ | $34.79^{a}$ | 0.80 | Linear | 0.0003 |

MBF: mesquite bean flour / SEM: standard error of mean / Different letters on the same line are significantly different by Tukey's test ($p<0.05$)

[1] $y=-1.346x+184.8$; $R^2=0.42$

[2] $y=0.2126x+23.549$; $R^2=0.48$

reduced some metabolites and caused direct mobilization of plasma triglycerides and liver glycogen ($p<0.05$). There was a reduction in glycogen levels, at 52.14% of substitution, indicating the occurrence of glycogenolysis (Fig. 3).

Elevation of the plasma concentration of free amino acids was observed and increased at 46.24% of corn substitution by MBF (Fig. 4).

Among the enzymes involved in nitrogen metabolism (Table 8), GDH stability was observed, while the hepatic and muscular ALTs and ASTs had reduced activities ($p<0.05$) depending on the treatments.

The LRP models indicate that initially the hepatic and muscular ALTs remained stable until 59.55 and 36.45% of corn substitution by MBF, respectively. From these points the enzyme activity showed a negative linear effect (Fig. 5).

**Table 8** Enzymatic activity of protein metabolism in Nile tilapia fed diets with corn substitution by mesquite bean flour ($n=8$)

| Variable[1] | Corn substitution by MBF (%) | | | | | | SEM | Effect | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | | | |
| *Liver* | | | | | | | | | |
| GDH | $7.65^{a}$ | $7.58^{a}$ | $7.33^{a}$ | $7.17^{a}$ | $6.88^{a}$ | $6.58^{a}$ | 0.15 | - | 0.2712 |
| ALT | $41.43^{ab}$ | $41.61^{ab}$ | $41.92^{a}$ | $46.18^{a}$ | $24.50^{bc}$ | $13.04^{c}$ | 2.65 | LRP | < .0001 |
| AST[2] | $88.12^{a}$ | $71.13^{a}$ | $50.33^{c}$ | $70.26^{ab}$ | $52.61^{bc}$ | $15.12^{d}$ | 4.53 | Linear | < .0001 |
| *Muscle* | | | | | | | | | |
| ALT | $45.65^{ab}$ | $45.69^{ab}$ | $54.71^{a}$ | $37.93^{b}$ | $38.29^{b}$ | $23.84^{c}$ | 2.12 | - | < .0001 |
| AST[3] | $158.15^{a}$ | $144.20^{a}$ | $99.58^{b}$ | $99.64^{b}$ | $92.79^{b}$ | $63.89^{c}$ | 6.39 | Linear | < .0001 |

MBF: mesquite bean flour / [1]U mg-1 protein / GDH: Glutamate dehydrogenase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase. SEM: standard error mean

Different letters on the same line are significantly different by Tukey's test ($p<.05$)

[2] $y=-2.2206x+92.346$; $R^2=0.62$

[3] $y=-3.4155x+162.65$; $R^2=0.74$

**Table 9** Hematological parameters in Nile tilapia fed diets with corn substitution by mesquite bean flour ($n=8$)

| Parameter[1] | Corn substitution by MBF (%) | | | | | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | | |
| Hb (g $dL^{-1}$) | 18.51[a] | 19.66[a] | 18.37[a] | 18.20[a] | 20.01[a] | 19.74[a] | 0.27 | 0.1952 |
| Hct (%) | 34.38[a] | 35.50[a] | 36.13[a] | 35.25[a] | 39.13[a] | 36.13[a] | 0.42 | 0.0224 |
| RBC ($10^6$ $\mu L^{-1}$) | 8.99[a] | 8.61[a] | 9.48[a] | 7.52[a] | 10.26[a] | 7.87[a] | 0.29 | 0.0497 |
| MCV (fL) | 40.00[a] | 42.66[a] | 40.02[a] | 48.57[a] | 39.38[a] | 46.89[a] | 1.35 | 0.2187 |
| MCH (pg) | 21.76[a] | 23.61[a] | 20.45[a] | 24.99[a] | 20.34[a] | 25.67[a] | 0.79 | 0.2130 |
| MCHC (g $dL^{-1}$) | 54.17[a] | 55.62[a] | 50.93[a] | 61.63[a] | 51.06[a] | 54.84[a] | 0.76 | 0.2900 |

MBF: mesquite bean flour / [1]Hb: Hemoglobin; Hct: Hematocrit; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration / SEM: Standard error mean

Different letters on the same line are significantly different by Tukey's test ($p<.05$)

### Hematology

No differences were observed between treatments for hemoglobin (g $dL^{-1}$), erythrocytes ($\times 10^6$ $\mu L^{-1}$), MCV (fL), MCH (pg), and MCHC (g $dL^{-1}$) (Table 9), indicating the same state of health among the animals.

## Discussion

Digestibility is an important aspect in the evaluation of alternative ingredients (Suprayudi, 2015). ADC represents the percentage of sample that is absorbed from the intestine of an animal (Xiong, 2014). In the present study, mean protein ADCs and MBF amino acids were above 80%. However, these values were less than those reported by Vidal, Xavier, Michelato, Martins, Pezzato, and Furuya (2015), when studying corn and its co-products in extruded diets for juveniles of Nile tilapia (101.6 ± 3.1 g). On the other hand, Vidal, Xavier, Moura, Michelato, Martins, and Furuya (2017) obtained high digestibility for proteins and amino acids and low digestibility for the energy of co-products derived from wheat in Nile tilapia (30.4 ± 4.6 g). Haidar, (2016) observed that ADC levels increased by 56% using non-starch polysaccharides for *Oreochromis niloticus*.

In the present study, ADC (%) was determined for lysine (82.9), methionine (82.3), cystine (82.3), and threonine (82.3). Vidal, Xavier, Michelato, Martins, Pezzato, and Furuya (2015) determined ADC (%) for lysine (88.8), methionine (95.3), cystine (99.6), and threonine (86.1), which were higher in corn that those determined for MBF. De Souza, Silva, Felix e Silva, Campeche, Melo, and Vidal (2019) determined ADC similar to that of the present study when studying the mesquite bean meal (MBM) in diets for Nile tilapia. The distinct processing did not change the

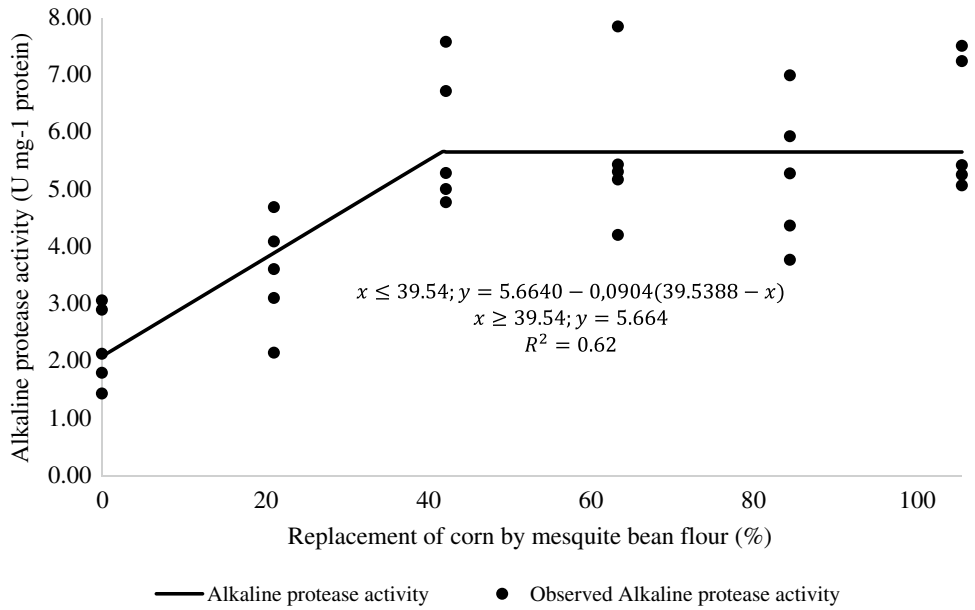


**Fig. 1** Activity of the digestive alkaline protease in Nile tilapia, fed diets containing different substitution levels of corn by mesquite bean flour (n=6)

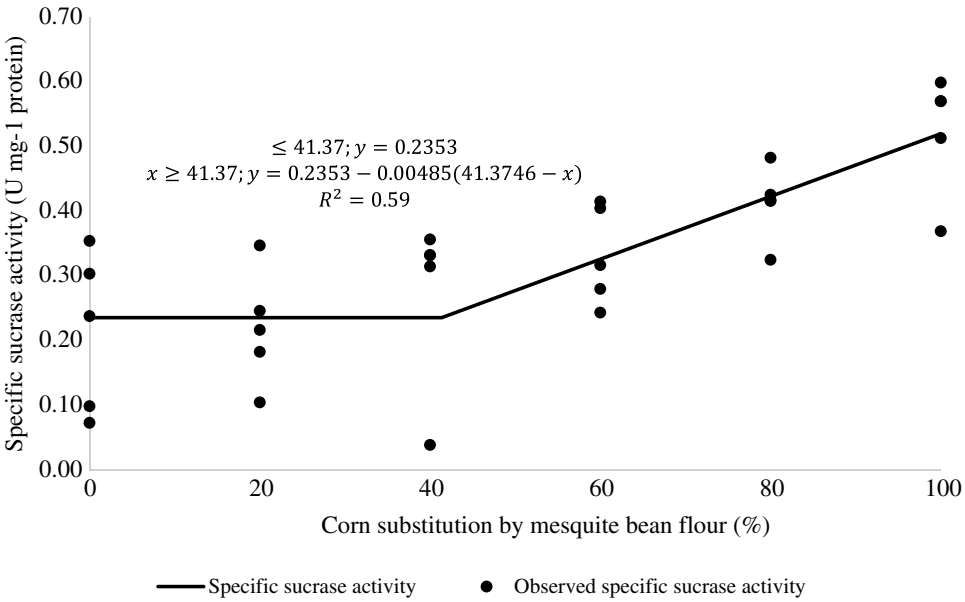


**Fig. 2** Specific activity of the digestive sucrose in Nile tilapia, fed diets containing different substitution levels of corn by mesquite bean flour (n=6)

amino acid and energy ADCs. Diets are formulated with large proportions of concentrated energy, reflecting substantial amounts of these amino acids from non-protein ingredients (NRC 2011).

Carbohydrates are the primary sources of energy in feed diets, being abundant in cereals, especially in corn and (NRC 2011). Starch is the main non-fibrous polysaccharide of corn; however, some monosaccharides and disaccharides predominate as energy source in alternative ingredients used in aquaculture (Hertrampf and Piedad-Pascual 2000). Tilapia has a high capacity for digesting gelatinized starch (Amirkolaie et al. 2006). The present study demonstrated adaptability and showed that sucrose was digested more efficiently than starch, based on the ADC (%) of starch (87.6) and sucrose (99.2). Starch is a complex polysaccharide that is digested by α-amylases, resulting in glucose monomers. Sucrose is a disaccharide that is the substrate of sucrase, which hydrolyses the α and β bonds (1–2) of sucrose to release glucose and fructose monomers (Nelson and Cox 2012). It is noted that exchange of sources results in different carbohydrate monomers whose transport mechanisms, metabolism, and absorption are different for each molecule (Pérez-Jiménez, 2015).

The replacement of corn by MBF did not cause changes in the zootechnical indexes evaluated. Other energetic ingredients have replaced corn in diets for Nile tilapia, maintaining

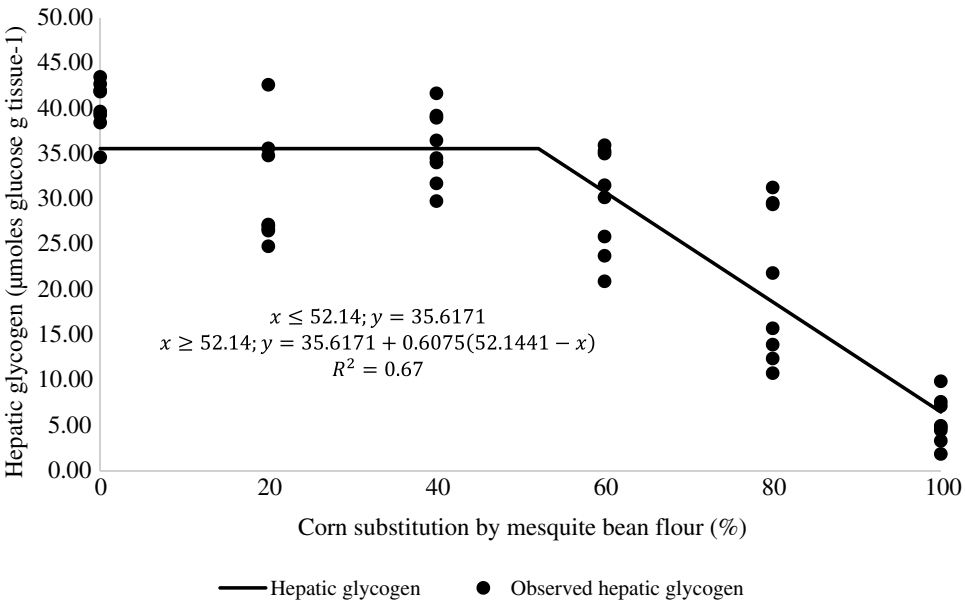


**Fig. 3** Hepatic glycogen of Nile tilapia, fed diets containing different substitution levels of corn by mesquite bean flour (n=8)

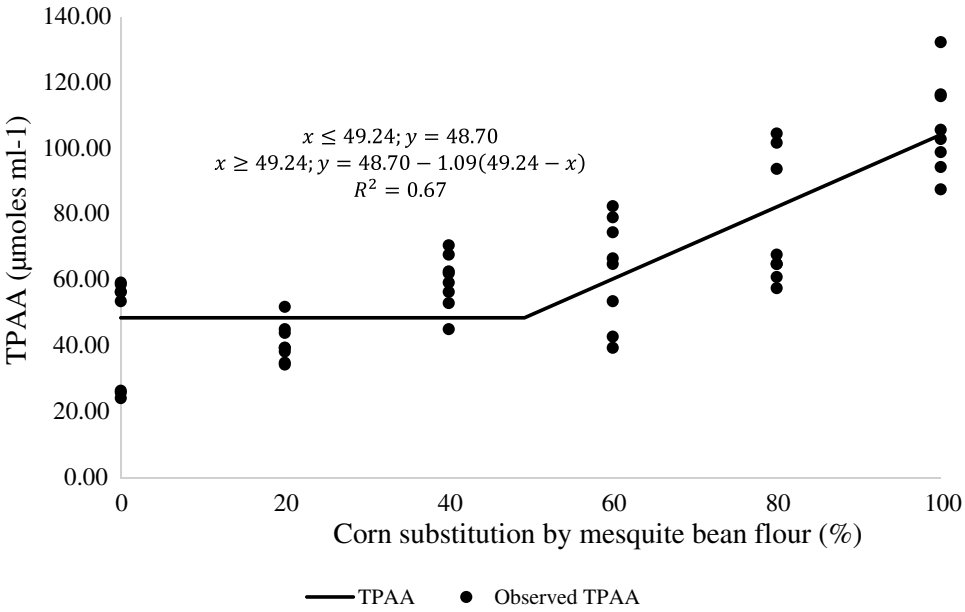


**Fig. 4** Total plasma amino acids from Nile tilapia, fed diets containing different substitution levels of corn by mesquite bean flour (n=8)

fish zootechnical indexes, including winter wheat (Signor, 2007) and triticale (Tachibana, 2010). On the other hand, De Souza, Silva, Felix e Silva, Campeche, Melo, and Vidal (2019) indicated that only 20% of corn could be replaced with MBM, due to the reduction of feed intake and feed conversion, possibly due to the presence of phenol compounds.

In this study, the predominance of sucrose digestion could be observed. The MBF has different concentrations of sucrose and starch (587.0 and 121.0 g $kg^{-1}$), and the ratio of starch/sucrose suggests an increase the concentration of the substrate. However, there are other factors that affect starch digestibility in fish including origin (plant species), physical condition (degree of gelatinization, complex or molecular weight), and inclusion levels in the diet (Polakof, 2012).

Amylase showed homogeneous activity, which may indicate an optimization of carbohydrases in the digestion process. Amylase activity was not changed due to the different starch concentrations between MBF (Table 2) and corn (Boonanuntanasarn, 2018), suggesting that the enzyme afforded plasticity. The concentration of a nutrient directly influences the secretion of digestive enzymes. In some cases, the low concentration of nutrient or low amount of ingredient results in increased enzyme secretion for greater digestive and absorptive efficiency (Polakof and Panserat 2016).

Alkaline protease showed the highest activity with the D100 diet ($p < 0.05$), leaving more free amino acids in the plasma. On the other hand, plasma amino acid oxidative routes (gluconeogenesis) were decreased as can be seen by

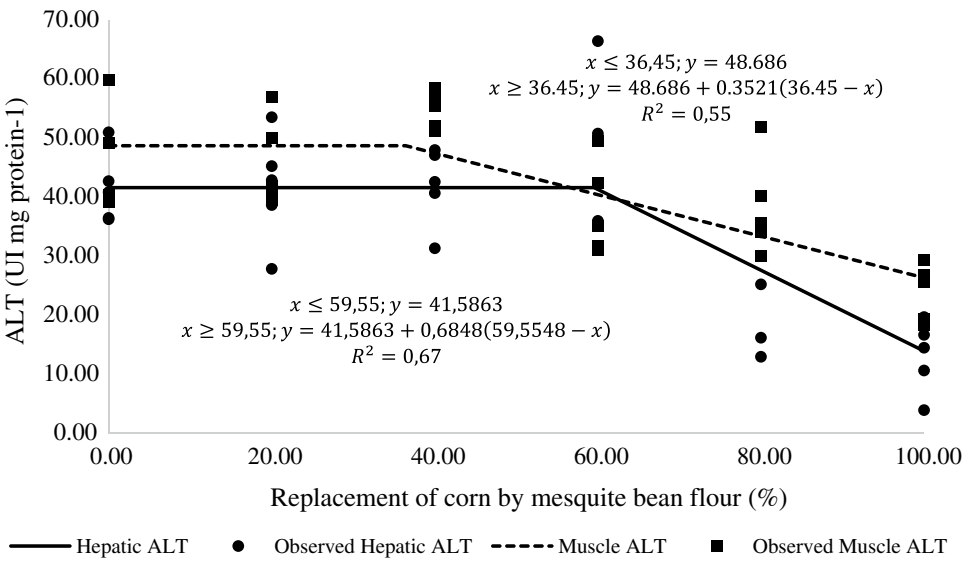


**Fig. 5** Specific activity of the metabolic enzyme alanine aminotransferase (liver and muscle) in Nile tilapia, fed diets containing different substitution levels of corn by mesquite bean flour (n=8)

the reduction of transamination enzymes (ALT and AST). These metabolic findings may indicate that the greater inclusion of MBF reduced the use of amino acids in gluconeogenesis, consequently a protein-sparing effect (Bureau et al. 2002).

Other studies have already evaluated the substitution of starch for monosaccharides and disaccharides and indicate different results. Nile tilapia juveniles fed semipurified diets in which starch was completely replaced by mono and disaccharides, showed reduced growth and protein deposition efficiency (Shiau and Chuang 1995; Shiau 1997; Shiau and Peng 1993). On the other hand, Azaza et al. (2020) fed juvenile Nile tilapia with practical diets in which part of the total starch (supplemented + starch from the ingredients) was replaced by mono and disaccharides. These authors observed a similar performance between treatments containing only starch and partial replacement treatments with maltose and dextrose. Among the hematological and metabolic variables, treatment with dextrose was similar to treatment with starch alone. In the present study, we used practical diets, so the experimental conditions were similar to that reported by Azaza et al. (2020). This may indicate that a good starch/sucrose ratio may have a beneficial effect on fish metabolism. This may be a result of a balance between the slower supply of glucose (due to the degradation of starch) and the availability of fructose (from sucrose) which, as an intermediate in glycolysis, can accelerate the ATP formation. This process generates an energetic positive balance, inhibiting gluconeogenesis (Dabrowski and Guderley 2003; Nelson and Cox 2012).

In the present study, tilapia adaptability was observed in using a type of carbohydrate more soluble than starch. The opposite situation was also observed when starch was replaced by a less soluble type of carbohydrate, the non-starch polysaccharides, there was no change in the digestibility of diets and zootechnical indexes of Nile tilapia juveniles (Haidar, Petie, Heinsbroek, Verreth and Schrama 2016).

Replacement of corn by MBF can optimized the use of dietary protein for growth purposes, as the diets were isonitrogenous and the amino acid profile remained mostly stable among treatments. Adequate supply and type of carbohydrates in the diet is important because they reduce the catabolism of proteins for energy and gluconeogenesis, which increases protein retention and decreases the excretion of ammonia to the environment (Rito, 2019).

Regarding the metabolic profile, it was observed that glucose was changed upon replacement of corn with MBF. There are several factors that alter blood glucose levels including nutritional, hormonal, physiological, and behavioral factors (Zhang, 2019). The effects of these factors were minimized in this study, and only variation in the nutritional factors was observed. The glucose homeostasis observed in the fish diets D0 and D100 allowed constant glucose utilization available for metabolism and energy production by the cells. These levels are in the normal range for the species (Weinert et al. 2015).

One of the metabolites that contributes to maintenance of blood glucose is hepatic glycogen, which was reduced at 52.14 (%) of its concentration in the corn substitution by MBF ($p < 0.05$). Liver glycogen degradation occurs via glycogenolysis, which leads to the formation of glucose-1-phosphate and then glucose-6-phosphate, which is cleaved to produce free glucose in circulation (Enes, 2010). Reduction of this hepatic reserve is related to the ability to mobilize liver glycogen for glucose maintenance as noted in *Dentex dentex* (Pérez-Jiménez, 2009), as well as in a food restriction study on *Sparus aurata* (Pérez-Jiménez et al. 2009) and *Acipenser baerii* (Zhu, 2011).

Corn substitution by MBF promoted better utilization of dietary protein. In *Oreochormis niloticus*, reductions in liver activity (ALT and AST) were observed with diets based on vegetable sources and it was suggested that this reduction occurs by distancing the nitrogenous products of the oxidative pathways (Schrama, Haidar, Geurden, Heinsbroek, and Kaushik 2018). On the other hand, Gaye-Siessegger et al. (2006) observed that high protein/starch ratios increased the ALT and AST activities in juveniles of *Oreochormis niloticus*. These studies denote that nutrients and feed sources can increase and/or decrease the deamination and transamination in fish. The increase in muscle glycogen in tilapia as caused by the MBF may be indicative of improved meat quality. The greater presence of glycogen in muscle causes a greater decrease in muscle pH during the onset of rigor mortis, thus ensuring better organoleptic characteristics and greater shelf life for the final product (Gagaoua, 2016). Therefore, replacement of corn with mesquite promotes a metabolic profile involving the use of carbohydrate sources for oxidative purposes and storage of carbohydrate reserves in the muscles.

The inclusion of MBM in tilapia diets reduced plasm total plasma amino acids (TPAA) levels (De Souza, Silva, Felix e Silva, Campeche, Melo and Vidal 2019). The present study detected an opposite situation, at 49.84% of corn substitution by MBF, corresponding to 14.77% of inclusion of MBF in the diet. This parameter indicates that more amino acids are free in the circulation, consequently available for deposition in tissues or other metabolic functions. The diets of this experiment were formulated to meet the digestible amino acids nutritional requirements of Nile tilapia (Furuya, Pezzato, Barros, Boscolo, Cyrino, Furuya and Feiden 2010); thus, the greater availability of amino acids did not benefit fish growth (NRC 2011). In theory, given the sparing capacity observed in the ingredient, the use of MBF in the formulations could allow the reduction of the protein indices of the diets, maintaining the growth rates.

In the present study, the substitution of corn by MBF did not influence the hematemesis indexes ($p > 0.05$). These values are similar to those reported by Tavares-Dias et al. (2003) for hemoglobin (17.6 g dL-1), CHCM (50.4%), and hematocrit (32.9%) even though the mean values were higher, indicating that hemoglobin levels, CHCM, and hematocrit in this study are within the range observed for the species. The constancy in hematological parameters indicates that substitution of corn by MBF did not cause hemodilution, hemoconcentration, or any imposed metabolic changes that could compromise the hematological profile and did not result in anemia or reduction in circulating red cells that is usually indicative of infectious diseases or malnutrition (Tavares-Dias and Moraes 2003).

## Conclusions

MBF has high digestibility for nutrients and energy and caused induction of digestive enzymes. Corn replacement by MBF did not result in differences in zootechnical variables and provided more total plasmatic amino acids and less transamination, which could indicate a protein-sparing effect. The hematological variables were not altered, indicating normality of health and nutritional status. Therefore, MBF can replace 100% of the corn in diets for Nile tilapia.

**Authors' contributions** A.M. de Souza and L.V.O. Vidal performed the project, conducted the trials, and wrote the draft. A.F. e Silva, D.F.B. Campeche, and J.F.B. Melo helped throughout the experimental period at UNIVASF. A.T.S. dos Santos assisted in the final technical and linguistic revisions of the manuscript.

**Funding** The authors acknowledge support from the "Fundação de Amparo à Pesquisa do Estado da Bahia" – Bahia – Brasil (FAPESB), grant term RED0027/2014 and "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" – Brasil (CAPES).

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

**Code availability** Not applicable.

## Declarations

**Ethics approval** This study was conducted in strict compliance with the Brazilian legislation for research and experimentation with animals and was approved by the Committee of Ethics in the Use of Animals of the Federal University of Bahia, located in Salvador, BA, Brazil (no. 09/2016).

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare that they have no competing interests.

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