



Palm kernel meal (*Elaeis guineensis*) as a substitute for corn (*Zea mays*) in diets of Tambaqui (*Colossoma macropomum*)

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Abstract

Two experiments were carried out to evaluate the effects of palm kernel meal (*Elaeis guineensis*) as corn (*Zea mays*) substitutes in tambaqui (*Colossoma macropomum*) diets based on zootechnical performance, health, characteristics of the muscle, yield and economic efficiency. The first one was carried out in a recirculation aquaculture system for 75 days wherein tambaquis received diets with 0% (T0), 25% (T25), 50% (T50), 75% (T75) and 100% (T100) of corn replacement by palm kernel meal. The second experiment, conducted in ponds for 184 days, tambaquis were fed T0 and T25 diets (selected because showed greater weight gain without health damage). Final weight and weight gain of tambaquis were impaired by the increasing levels of this ingredient on diets, and the feed conversion ratio was worse. The protein efficiency ratio and specific growth rate gradually reduced, while the total number of leucocytes and neutrophils gradually increased. Cholesterol and triglycerides decreased in T75 and T100. Variables analysed in experiment 2 were not affected by treatment, except muscle lipids. Palm kernel meal can replace up to 25% of corn in tambaqui diets since it maintains a balance in economic and zootechnical performance of production.

KEYWORDS

fish nutrition, palm oil by-product, Tambaqui, zootechnical performance

1 | INTRODUCTION

Corn (*Zea mays* L.) is the main energetic ingredient used both in animal feeds (Hasan & New, 2013) and in human diets. Market corn demand makes animal feed industries to search for alternative ingredients with low prices, continuous supply, established manufacturing process and scale production.

Palm kernel (*Elaeis guineenses* Jacq.) is an alternative source, economically feasible, currently used as raw material to produce biofuel and cooking oil (Mahlia, Ismail, Hossain, Silitonga, & Shamsuddin, 2019). During the extraction process, some by-products are obtained: the palm kernel cake from the screw pressing and the palm kernel meal

(PKM), by oil solvent extraction (Heuzé et al., 2016), in this case, hexane, for an hour. In 2018, about 9.8 million tons of PKM were commercialized in world markets (Index Mundi, 2018), a large amount that justifies the need to increase the options for using this by-product.

There are no reports of toxicity of PKM in fish (Yinhui, Meng, Xiguang, & Fangkui, 2008) and, in spite of high crude fibre, it can be included in fish feeds preparations as sources of energy or protein, provided it is included in adequate proportions considering the species tolerance (Hertrampf & Piedad-Pascual, 2000). In general, the safe inclusion of palm oil by-products in feeds for *Oreochromis niloticus* (Adjanke, Tona, Ble, Toko, & Gbeassor, 2016; Carvalho, Azevedo, Ramos, & Braga, 2012; Yinhui et al., 2008), *O. niloticus* × *O. mossambicus* (Iluyemi, Hanafi,

Radziah, & Kamarudin, 2010), *Chanos chanos* (Zulfahmi et al., 2019), hybrid Asian-African catfish *Clarias macrocephalus* × *C. gariepinus* (Ng & Chen, 2002) and *Labeo senegalensis* (Omoriegie, 2001) provided good zootechnical performance. However, the effects of PKM in the so-called round fish diets, such as tambaqui, are not well understood.

Tambaqui (*Colossoma macropomum*, Cuvier, 1818) is the major round fish species farmed in Brazil and represents 18.2% of the Brazilian production (IBGE, 2017a). Its omnivorous feeding habit (Wojnárovich & Anrooy, 2019) provides digestive plasticity for efficient utilization of vegetable-based diets. Considering the high availability of PKM and its low cost, there is a research interest in evaluating the effects of increasing inclusion in tambaqui diets to support safe recommendations in aquafeeds. Therefore, the objective of this study was to investigate these effects of PKM as a substitute for corn in tambaqui diets based on zootechnical performance and physiological aspects, physical and chemical characteristics of muscle, yield and economic efficiency under experimental conditions.

2 | MATERIALS AND METHODS

Two sequential experiments were approved by the Commission on the Ethical Use of Animals (CEUA) of Embrapa Eastern Amazon (Protocol no. 006/2016).

2.1 | Preparation of the diets

The ingredients were ground in a hammer mill with a 0.5 mm mesh, weighed and homogenized. The mixtures were pelletized with matrices of 2 mm (experiment 1) and 2–6 mm (experiment 2), dried in a forced air circulation oven (55°C) for 24 hr and stored in a freezer (–23°C) until use.

2.2 | Experiment 1

Five experimental diets containing 0%, 25%, 50%, 75% and 100% corn replacement by PKM were formulated (Table 1). The experimental diets attended nutritional requirements of this species (Lima, Bonfim, Siqueira, Ribeiro, & Lanna, 2016).

2.2.1 | Fish and experimental conditions

The fish were initially acclimated for 10 days to laboratory conditions, and after this period, 120 individuals (16.35 ± 0.61 g) were distributed into 15 circular tanks (200 L) in a recirculating aquaculture system (RAS) with continuous aeration. The water quality was monitored using a digital meter measuring dissolved oxygen (7.6 ± 0.2 mg/L), temperature ($29.1 \pm 0.8^\circ\text{C}$) and pH (6.2 ± 0.2), and total ammonia (0.36 ± 0.2 mg/L), nitrite (0.05 ± 0.02 mg/L) and

nitrate (6.4 ± 1.1 mg/L) were analysed using colorimetric methods, maintained within the optimum range (Aride, Roubach, & Val, 2007).

The experiment was conducted for a period of 75 days in a completely randomized design with five treatments (T0, T25, T50, T75 and T100) and three replicates. The fish were fed four times a day up to satiation.

At the end of the experiment, all the fish were weighed and the following parameters were calculated: final weight (FW, g); weight gain (WG, g) = final weight – initial weight; feed conversion ratio (FCR) = feed consumption (g)/ weight gain (g); specific growth rate (SGR, %/day) = $[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{ experimental period}] \times 100$; protein efficiency ratio (PER, %) = (weight gain (g)/ crude protein consumption (g)) × 100; and survival (%) = (final number of fish/ initial number of fish) × 100.

At the end of experiment 1, three fish from each experimental unit were randomly selected for blood and muscle sampling by an incision made between the skull and the first vertebra.

2.2.2 | Haematological and biochemical analyses

Blood collection was done by puncturing the caudal vessel using heparinized syringes, and haematological variables were measured using total blood samples. Red blood cells were counted in a Neubauer chamber, haemoglobin concentration was evaluated using the Drabkin reagent with a spectrophotometer (Biospectro SP-220, Curitiba, Brazil) reading made at an absorbance of 540 nm, and haematocrit values were determined using the microhaematocrit method (Ranzani-Paiva et al., 1999). With these data, the following indices were calculated according to Wintrobe (1934): mean corpuscular volume (MCV, fL) = $[(\text{haematocrit} \times 10) / (\text{number of red blood cells} \times 10^6 \mu\text{l}^{-1})]$, mean corpuscular haemoglobin (MCH, g/dl) = $[(\text{haemoglobin concentration} \times 10) / (\text{number of red blood cells} \times 10^6 \mu\text{l}^{-1})]$ and mean concentration of corpuscular haemoglobin (MCHC, g/dl) = $(\text{haemoglobin concentration} \times 100) / \text{haematocrit}$.

The total count and the difference in leucocytes and the total of thrombocytes were conducted using blood smears coloured with May–Grünwald–Giemsa–Wright stain (Ranzani-Paiva et al., 1999). Plasma was separated through centrifugation at $1.800 \times g$ for 5 min and then stored at -20°C . The plasma samples obtained were used to determine the concentrations of glucose, total proteins, cholesterol and triglycerides using colorimetric kits (Doles®), and absorbance readings were performed using a spectrophotometer (Biospectro SP-220).

2.3 | Experiment 2

This experiment measured the effect of the diet that provided the best zootechnical performance without any health changes in experiment 1 (T25) compared to the control (T0) under field conditions.

TABLE 1 Formulation and proximate composition of experimental diets and ingredient (g/kg dry matter) utilized in the experiment 1 and in the experiment 2 (T0 and T25)

Ingredients	Experimental diets					Ingredient
	T0	T25	T50	T75	T100	PKM ^a
Corn	250.3	187.8	125.0	62.5	00.0	
PKM ^a	00.0	62.6	125.0	187.5	250.1	
Soybean meal	480.0	481.8	465.0	448.0	431.1	
Wheat meal	00.0	08.0	25.0	42.0	58.2	
Fishmeal	211.7	202.8	202.5	202.5	202.6	
Soybean oil	27.0	26.0	26.5	26.5	27.0	
Dicalcium phosphate	10.0	10.0	10.0	10.0	10.0	
DL-Methionine	03.0	03.0	03.0	03.0	03.0	
L-Lysine	13.0	13.0	13.0	13.0	13.0	
Premix ^b	05.0	05.0	05.0	05.0	05.0	
Proximate composition						
Moisture	74.1	82.7	78.7	79.0	98.9	129.7
Crude protein	355.7	352.0	354.4	351.9	350.3	140.8
Crude lipid	55.2	52.9	40.2	42.1	42.7	15.2
Ash	97.4	95.8	97.0	99.2	104.1	38.8
ADF ^c	71.9	79.7	114.5	141.3	186.4	364.0
NDF ^d	355.7	380.2	422.7	436.3	476.7	511.4
Crude fibre	-	-	-	-	-	212.2
Gross energy (MJ/kg) ^e	17.72	17.53	17.33	17.38	16.87	17.27

^aPalm kernel meal supplied by Denpasa (Dendê do Pará S/A), Pará, Brazil

^bvitamin and mineral premix Vitamitract, Chapecó, Brazil (kg⁻¹): potassium 2,000.00 mg; magnesium 600 mg; copper 1,000.00 mg; iron 7,500.00 mg; zinc 7,500.00 mg; manganese 2,000.00 mg; selenium 70.00 mg; iodine 250.00 mg; cobalt 30.00 mg; choline 80.00 g; vitamin A 2,000,000.00 UI; vitamin D3 600,000.00 UI; vitamin E 15,000.00 UI; vitamin K3 700.00 mg; nicotinic acid 10.00 g; pantothenic acid 5,000.00 mg; folic acid 100.00 mg; biotin 50.00 mg; vitamin B1 2,000.00 mg; vitamin B2 4,000.00 mg; vitamin B6 5,000.00 mg; vitamin B12 10,000.00 mg; vitamin C 80.00 g; inositol 4,000.00 mg; and ethoxyquin 1,000 mg; B.H.T. 5,000 mg

^cacid detergent fibre

^dneutral detergent fibre

^egross energy as calculated using the factor stated by NRC (2011): Crude protein × 5.64 + Crude lipid × 9.44 + Nitrogen-free extract × 4.11.

2.3.1 | Animals and experimental conditions

Two hundred and forty tambaquis (15.52 ± 1.84 g), previously acclimated for 10 days to experimental conditions, were distributed into six ponds (48.50 m²), protected by shade screen (18%) and with constant renovation of water. The experiment was conducted for a period of 184 days using a completely randomized design with two treatments (T0 and T25) and three replicates, with each pond being an experimental unit. The fish were fed four times a day until satiety, and at the end of the experiment, all fish from each pond were weighed and four fish from each experimental unit were randomly selected and euthanized by an incision made between the skull and the first vertebra, for muscle sampling, and twenty fish were randomly selected for yield and growth performance measurements (FW, WG, FCR, SGR, PER).

The water quality parameters were monitored and maintained within the optimum range: dissolved oxygen, 6.3 ± 1.5 mg/L; temperature, 28.6 ± 0.5 °C; pH, 6.0 ± 0.6; conductivity, 53.97 ± 10.08

µS/cm; total ammonia, 0.6 ± 0.8 mg/L; nitrite, 0.01 ± 0.01 mg/L; and nitrate, 0.3 ± 0.4 mg/L.

2.3.2 | Economic analysis

Prices for inputs were based on those found in retail outlets in the mesoregion of the metropolitan area of Belém, Pará, Brazil. Using these prices as a base, the price of 1 kg of each diet was US\$ 0.47 and US\$ 0.46 for the T0 and T25 treatments respectively. The cost of feed per kg of live weight gained was calculated according to Bellaver, Fialho, and Protas (1985): feed cost (CR, US\$/kg) = (quantity of experimental diet (kg) × price kg of diet)/ weight gain (kg). The indices of economic efficiency and cost were calculated according to Fialho, Barbosa, Ferreira, Gomes, and Giroto, (1992): Economic Efficiency Index (EEI, %) = (MCE/ CTei) × 100; and Cost Index (CI, %) = (CTei/ MCE) × 100, where: MCE = lowest cost of a diet; and CTei = cost of a diet for the treatment i.

TABLE 2 Growth performance (mean ± SE) of tambaqui fed diets for 75 days in the experiment 1 and for 184 days in the experiment 2

Items	Experiment 1					Experiment 2				
	T0	T25	T50	T75	T100	p value	T0	T25	T50	p value
FW (g)	111.5 ± 1.92 ^{ab}	144.5 ± 5.90 ^a	102.7 ± 9.06 ^b	102.0 ± 6.44 ^b	95.6 ± 13.32 ^b	.0126	813.69 ± 32.56	807.67 ± 46.22	807.67 ± 46.22	.9203
WG (g)	95.3 ± 2.31 ^{ab}	127.6 ± 5.89 ^a	86.5 ± 8.71 ^b	85.9 ± 6.60 ^b	79.6 ± 13.16 ^b	.0131	798.19 ± 32.58	792.17 ± 46.22	792.17 ± 46.22	.9203
FCR	1.17 ± 0.06 ^b	1.24 ± 0.03 ^b	1.25 ± 0.05 ^b	1.39 ± 0.03 ^{ab}	1.47 ± 0.06 ^a	.0074	1.04 ± 0.05	1.02 ± 0.01	1.02 ± 0.01	.7316
PER (%)	2.41 ± 0.12 ^a	2.29 ± 0.05 ^{ab}	2.28 ± 0.10 ^{ab}	2.01 ± 0.05 ^b	1.95 ± 0.08 ^b	.0114	2.72 ± 0.13	2.80 ± 0.04	2.80 ± 0.04	.5961
SGR (% day ⁻¹)	2.57 ± 0.06 ^{ab}	2.86 ± 0.52 ^a	2.45 ± 0.10 ^{ab}	2.46 ± 0.10 ^{ab}	2.33 ± 0.16 ^b	0.0395	2.15 ± 0.02	2.15 ± 0.03	2.15 ± 0.03	.8790

Abbreviations: FW, final weight; WG, weight gain; FCR, feed conversion ratio; PER, protein efficiency ratio; SGR, specific growth ratio. Values in the same row with different superscript letters are significantly different ($p < .05$).

The cost for fry was estimated at US\$ 0.07 for each unit, including shipping and handling, and the value of US\$ 1.84 per kg of live weight was used for the sale of fish (*in natura*) at the place of production. The estimation of costs was done following the method proposed by Silva, Kronka, Sipaúba-Tavares, Silva-Junior, and Souza (2003): gross revenue (GR, US\$) = total biomass produced × price for the sale of 1 kg of fish; partial operational cost (POC, US\$) = (Quantity of feed × price per kg of feed) + (initial number of fry × fry unit price); and partial net revenue (PNR, US\$) = gross revenue – partial operational costs.

2.3.3 | Physical and chemical characteristics of muscle and yield

The proximate composition of the muscle was determined along with the pH using a bench pH meter (HANNA Instruments, HI-221, Woonsocket, USA), and the colour was measured with a colorimeter (Konica Minolta Sensing Inc, CR-400, Sakai, Japan) according to the CIELAB system (CIE, 1986), defined by L* (luminosity) and the chromaticity coordinates a* and b* that vary from green (-) to red (+) and blue (-) to yellow (+) respectively.

To evaluate yield, each fish (20 per pond) was considered an experimental unit, and measurements and per cent yield calculations were done using the gutted fish, clean body trunk, loin and ribs using the formula $\eta = (\text{final weight}/\text{initial weight}) \times 100$.

2.4 | Analytical methods

Ingredients, diets and muscle sampled in the experiments were analysed following the method of AOAC (2012). Moisture was determined by drying the sample in an oven (Tecnal TE-394/2MP, Piracicaba, Brazil) at 105°C until a constant weight, and crude protein (N 6.25), was measured using a Micro-Kjeldahl apparatus (Tecnal TE-036/1, Piracicaba, Brazil). Crude lipid was quantified through extraction using petroleum ether in a Soxhlet (Cientec CT-340, Belo Horizonte, Brazil) during 16 hr, and ash was quantified through combustion in a muffle furnace (Quimis Q318M24, Diadema, Brazil) at 550°C for 6 hr. Crude fibre was estimated according to Silva and Queiroz (2002), and acid detergent fibre and neutral detergent fibre were measured following the method in Van Soest (1967).

2.5 | Statistical Analyses

The results of experiment 1 were submitted to analysis of variance (one-way ANOVA) followed by Tukey's multiple comparisons test. When normality and homogeneity assumptions were not met, the Kruskal-Wallis test was used and statistical differences were detected by Dunn test. Differences among groups of experiment 2 were assessed by a *t* test. All analyses were performed using the SAS university edition statistical software (SAS, 2017) at 5% probability level.

3 | RESULTS

3.1 | Experiment 1

The survival rates were not affected by dietary treatment levels of PKM. Final weight (FW), the weight gain (WG) and the feed conversion ratio (FCR) of tambaquis were impaired by the increasing levels of this ingredient on diets of tambaquis. The protein efficiency ratio (PER) and specific growth rate (SGR) reduced gradually (Table 2; $p < .05$). The proximate composition of the muscle showed that body moisture, protein and ash did not show a clear effect, whereas lipids were reduced (Table 3; $p < .05$).

There were no significant differences in terms of haematocrit, haemoglobin, red blood cells, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean concentration of corpuscular haemoglobin (MCHC), thrombocyte count, lymphocytes, monocytes, eosinophils, PAS-positive granular leucocyte (PAS-GL) and glucose in treatments ($p > .05$). However, the total number of leucocytes and neutrophils gradually increased as an effect of treatments ($p < .05$). Cholesterol and triglycerides decreased in T75 and T100, and total protein increased in these treatments (Table 4; $p < .05$).

The T25 diet afforded the best WG without any changes on haematological and biochemical parameters, being chosen for evaluation in field conditions.

3.2 | Experiment 2

During this experiment, all dietary treatments had 100% survival. Zootechnical performance ($p > .05$; Table 2), physical and chemical composition of muscle, yield ($p > .05$; Table 5) and economic indicators ($p > .05$; Table 6) did not differ between treatments, except for the lipid content which decreased in T25 ($p < .05$; Table 5). Samples were considered fresh considering the pH of muscle.

4 | DISCUSSION

Adequate levels of dietary fibre can promote growth and improve immunological responses in animals (Yarahmadi, Kolangi Miandare, Farahmand, Mirvaghefi, & Hoseinifar, 2014). This effect may have favoured the growth of tambaquis in T25 (experiment 1), indicating

the tolerance of the species up to this replacement level. High fibre content in diets acts as an antinutrient, interferes in the assimilation of nutrients and reduces growth (NRC, 2011), which may justify the poor performance of tambaquis in T50 and T100.

The total carbohydrate composition of corn and PKM showed large differences because just 9.7% of corn carbohydrates are in a non-starch polysaccharide—NSP form, while PKM corresponds to more than 81% (Knudsen, 2001). Of this total, almost 96% is in an insoluble form—cellulose and hemicelluloses (Abdollahi, Hosking, & Ravindran, 2015; NRC, 2011). NSPs in food have already been related to the poor digestibility of the energy fraction in diets for tambaquis (Buzzolo et al., 2018; Guimarães, Miranda, & Araújo, 2014; Silva, Pereira Filho, & Oliveira-Pereira, 2003; Silva et al., 2019). This is because, in fish and other monogastrics, β -glucanases or β -xy-lanases, enzymes that degrade these carbohydrates, are scarce or nonexistent (Kuz'mina, 1996), preventing their use as an energy source (NRC, 2011). This reduced digestibility caused by NSPs has an effect on the growth, performance and health of animals (Bhatt, Chovatiya, & Shah, 2011; NRC, 2011).

FCR is an important indicator for fish culture and has a direct impact on the cost of production of a farmed species (Bureau, 2008). In experiment 1, the increase PKM insoluble fibre on diets (21.22% in PKM and 1.58% in corn—Table 1) impaired FCR and limited growth. The opposite was observed in experiment 2, with FCR in acceptable range (1.02–1.04) proportionated by diets up to 25% PKM.

Increasing substitution levels of corn by PKM in diets reduced PER (Table 2) indicating that, in excess (above T50), negatively influenced the absorption of dietary protein (Bhatt et al., 2011; NRC, 2011; Rodrigues, Fernandes, Fabregat, & Sakomura, 2010). Although fish do not have a specific nutritional requirement for carbohydrates, their appropriate inclusion in diets can spare the preferential use of proteins as energy source (NRC, 2011), enabling the formulation of low-cost diets.

The effect of the treatments on muscle composition showed that the presence of intramuscular spines did not increase the content of ash, which is consistent with the literature (Adjanke et al., 2016). However, the tendency of less mineral accumulation could have been caused by lignin, an insoluble carbohydrate, which exists in PKM (12.8%) (Heuzé et al., 2016) and has a strong capacity to form metallic ion complexes which inhibit mineral absorption by the intestinal epithelium (Kamalam, Medale, & Panserat, 2017).

TABLE 3 Proximate composition (mean \pm SE) of muscle in tambaquis fed diets for 75 days in the experiment 1 (% wet basis)

	Diets					p value
	T0	T25	T50	T75	T100	
Moisture	78.87 \pm 0.55 ^b	79.64 \pm 0.13 ^b	80.15 \pm 0.32 ^{ab}	79.56 \pm 0.09 ^b	81.64 \pm 0.35 ^a	.0018
Crude protein	17.84 \pm 0.04 ^{ab}	17.52 \pm 0.16 ^{ab}	16.80 \pm 0.29 ^b	18.30 \pm 0.39 ^a	16.60 \pm 0.35 ^b	.0108
Crude lipid	1.20 \pm 0.05 ^a	0.81 \pm 0.02 ^c	0.86 \pm 0.01 ^c	1.00 \pm 0.01 ^b	0.59 \pm 0.04 ^d	<.0001
Ash	1.32 \pm 0.02 ^a	1.12 \pm 0.03 ^b	1.11 \pm 0.03 ^b	1.25 \pm 0.21 ^{ab}	1.15 \pm 0.32 ^b	.0042

Abbreviations: Values in the same row with different superscript letters are significantly different ($p < .05$).

TABLE 4 Haematological and biochemical parameters (mean \pm SE) of tambaqui fed diets for 75 days in the experiment 1

	Diets					p value
	T0	T25	T50	T75	T100	
Haematocrit (%)	21.93 \pm 0.79	24.83 \pm 1.22	21.86 \pm 0.80	23.75 \pm 0.75	24.13 \pm 0.78	.2702
Hb (g/dl)	6.07 \pm 0.48	8.07 \pm 0.56	7.71 \pm 0.38	8.55 \pm 1.09	8.07 \pm 1.10	.2949
RBC ($10^6/\mu\text{l}$)	0.69 \pm 0.09	0.63 \pm 0.04	0.74 \pm 0.04	0.76 \pm 0.07	0.67 \pm 0.10	.5134
MCV (fL)	365.63 \pm 34.55	422.87 \pm 35.76	311.95 \pm 27.93	337.67 \pm 38.16	359.74 \pm 89.77	.6619
MCH (g/dl)	104.91 \pm 14.91	137.28 \pm 12.47	108.68 \pm 9.42	117.73 \pm 15.02	120.9 \pm 34.53	.7998
MCHC (g/dl)	27.65 \pm 1.59	34.91 \pm 3.26	35.54 \pm 1.22	35.69 \pm 3.64	36.33 \pm 2.13	.2610
Leucocytes ($10^3/\mu\text{l}$)	59.22 \pm 5.99 ^b	64.32 \pm 7.64 ^b	90.16 \pm 7.85 ^{ab}	119.06 \pm 13.23 ^a	129.90 \pm 69.08 ^a	.0191
Thrombocytes ($10^3/\mu\text{l}$)	8.57 \pm 0.58	11.53 \pm 1.53	17.08 \pm 2.42	17.44 \pm 2.11	19.99 \pm 11.27	.1451
Lymphocytes ($10^3/\mu\text{l}$)	30.24 \pm 2.53	42.92 \pm 6.31	52.12 \pm 4.78	57.86 \pm 7.17	67.94 \pm 35.84	.0663
Monocytes ($10^3 \mu\text{l}^{-1}$)	1.94 \pm 0.33	2.24 \pm 0.33	3.29 \pm 0.82	3.29 \pm 0.55	3.97 \pm 2.15	.2971
Neutrophils ($10^3/\mu\text{l}$)	25.61 \pm 4.00 ^b	29.12 \pm 2.55 ^{ab}	38.63 \pm 6.39 ^{ab}	53.7 \pm 5.22 ^a	53.14 \pm 24.38 ^{ab}	.0306
Eosinophils ($10^3/\mu\text{l}$)	0.64 \pm 0.18	0.52 \pm 0.15	0.73 \pm 0.29	0.91 \pm 0.41	1.96 \pm 1.70	.9940
PAS-GL ($10^3/\mu\text{l}$)	0.97 \pm 0.19	0.76 \pm 0.22	1.13 \pm 0.50	1.54 \pm 0.45	2.90 \pm 0.95	.4486
Glucose (mg/dl)	57.46 \pm 2.91	62.16 \pm 10.55	63.95 \pm 2.52	51.18 \pm 3.92	62.19 \pm 5.64	.1724
Total proteins (g/dl)	2.09 \pm 0.11 ^b	2.18 \pm 0.36 ^b	2.47 \pm 0.15 ^{ab}	3.20 \pm 0.26 ^a	3.09 \pm 0.26 ^a	.0002
Triglycerides (mg/dl)	225.96 \pm 15.99 ^{ab}	226.47 \pm 42.20 ^{ab}	258.88 \pm 15.27 ^a	121.71 \pm 8.89 ^c	197.12 \pm 14.21 ^b	<.0001
Cholesterol (mg/dl)	95.40 \pm 3.15 ^a	107.02 \pm 12.49 ^a	112.44 \pm 5.47 ^a	54.84 \pm 5.68 ^b	66.74 \pm 3.11 ^b	<.0001

Abbreviations: Hb, haemoglobin concentration; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PAS-GL, PAS-positive granular leucocyte. Values in the same row with different superscript letters are significantly different ($p < .05$).

The reduction in muscle lipids may have been caused both by the increase in dietary fibre, since it reduces the absorption of lipids by the intestine (Rodrigues et al., 2010) and in response to the fat content of diets.

There is a tendency towards an increase on white cells: lymphocytes, monocytes, PAS-GL and eosinophils, but which were not significant, probably due to the great variation in the values obtained. This intraspecific variation was reported for tambaquis and is normally expected between teleosts due to the migratory character of defence cells between the leucopoietic organs and the circulation system, and the responses to environmental stimuli to which each individual is subject (Tavares-Dias, 2015). There were alterations in the counts of leucocytes and neutrophils. Among the blood cells that present an organic defence for the teleosts, the neutrophils are highlighted since the greatest production of them occurs as an inflammatory response to an infectious process (Liongue, Hall, O'Connell, Crosier, & Ward, 2009), besides being sensitive to protein and energetic malnutrition (Flajnik & Du Pasquier, 2004). Therefore, it is possible that the elevation of neutrophils observed in T75 group,

with a tendency to increase in T100, and an increase in leucocytes in T75 and T100 (Table 4), probably indicates a nutritional pathology.

Diet composition may impact plasma triglycerides and total cholesterol levels (Panserat et al., 2017), which explains the lower values on T75 and T100 groups. NSPs form bonds with bile salts and inhibit the absorption of fats, and this increases the excretion of cholesterol in the faeces and reduces cholesterol circulating in the blood (Krögdahl, Hemre, & Mommsen, 2005). Low levels of plasmatic total cholesterol indicate a reduction in the antioxidant capacity of animals and greater susceptibility to infections, which can result in vascular diseases, neuropathy and nephropathy (Goulart et al., 2017) and lead to the higher numbers of leucocytes as neutrophils.

The freshness of samples from experiment 2 was based on legislation in force in the country (Brasil, 2017). The palm kernel has beta-carotene and alpha-carotene (Trigueiro & Pentead, 1996), which could have altered the colour of the muscle, but this effect was not observed. Gutted fish are widely commercialized in the Amazon region (Souza & Inhamuns, 2011). The gutted fish yield

TABLE 5 Proximate composition of muscle and yield (mean \pm SE) in tambaqui fed diets for 184 days in the experiment 2

	Diets		p value
	T0	T25	
Muscle			
Moisture (%)	80.28 \pm 0.10	80.74 \pm 0.37	.3111
Crude protein (%)	14.74 \pm 0.47	15.80 \pm 0.29	.1265
Crude lipids (%)	3.61 \pm 0.49 ^a	1.90 \pm 0.31 ^b	.0414
Ash (%)	0.88 \pm 0.05	0.87 \pm 0.05	.9618
pH	6.43 \pm 0.04	6.46 \pm 0.04	.5858
L*	55.96 \pm 1.31	54.63 \pm 1.77	.5682
a*	1.87 \pm 0.28	1.71 \pm 0.46	.7716
b*	13.05 \pm 0.61	12.40 \pm 0.56	.4612
Yield (%)			
Gutted fish	86.89 \pm 0.44	86.06 \pm 0.69	.3326
Clean body trunk	71.44 \pm 0.58	69.77 \pm 0.60	.0506
Loin	27.48 \pm 0.70	27.14 \pm 0.78	.7456
Ribs	19.32 \pm 0.71	19.01 \pm 0.47	.6529

Abbreviations: L*, luminosity; a* and b*, chromaticity coordinates; clean body trunk, without head, viscera, fins and scales. Values in the same row with different superscript letters are significantly different ($p < .05$).

(Table 5) was higher than that obtained in tambaqui from wild (83.43%; Souza & Inhamuns, 2011) and from fish culture (83.06%; Fernandes, Doria, & Menezes, 2010). In this species, the larger difference between gutted fish yield and clean body trunk is due to the head size, which is considered large in proportion to the body (Fernandes et al., 2010). In tambaqui, as well as in round fish, loin and ribs are considered premium cuts, being very appreciated (Marcus, 2015). Cartonilho and Jesus (2011) found a yield of 19.64% and 27.70% for tambaqui rib and loin, values close to those obtained in this research.

Aquaculture activities are very intensive in their use of production inputs, especially with respect to capital, labour, fries and feed. Due to this aspect, the determination of economic indicators is of fundamental importance because these will contribute to the efficient management and allocation of available economic resources with the goal of guaranteeing financial viability of the aquaculture activity (Engle, 2010).

Economic indicators show that a 25% corn replacement by PKM in feed is viable, and at certain times of the year when the price of corn is high, feed manufacturing industries can benefit from using PKM. Carvalho et al. (2012) evaluated four plant by-products in tilapia and reported a tendency of improvement in economic efficiency indices and costs when using palm kernel cake. In the specific case of the state of Pará (Brazil), the productive sector can benefit from the availability of this by-product as a function of the scale of production because it concentrates 97.5% of the national production of palm oil (IBGE, 2017b).

In this study, 25% corn replacement by PKM (6.26% of diet; Table 1) promoted better zootechnical performance without negatively altering the haematological and biochemical variables

TABLE 6 Economical parameters (mean \pm SE) of tambaqui fed diets for 184 days in the experiment 2

	Diets		p value
	T0	T25	
FC (US\$/kg)	0.49 \pm 0.02	0.47 \pm 0.01	.4818
EEL (%)	94.35 \pm 4.62	97.90 \pm 1.21	.4990
CI (%)	106.52 \pm 5.47	102.17 \pm 1.26	.4819
POC (US\$)	18.34 \pm 0.77	17.61 \pm 1.03	.6006
GR (US\$)	59.89 \pm 2.40	59.44 \pm 3.40	.9203
PNR (US\$)	41.55 \pm 2.17	41.83 \pm 2.37	.9340

Abbreviations: CI, Cost Index; EEL, Economic Efficiency Index; FC, feed cost per Kg live weight; GR, gross revenue; POC, partial operational cost; PNR, partial net revenue. Values in the same row with different superscript letters are significantly different ($p < .05$).

studied, thus corroborating the recommendation of inclusion at a rate of 5%–10% in diets of omnivorous fish (Hertrampf & Piedad-Pascual, 2000). In *C. chanos* diets, recommended substitution is up to 16.36% (Zulfahmi et al., 2019); for the hybrid *C. macrocephalus* \times *C. gariepinus*, up to 20% (Ng & Chen, 2002), while for *O. niloticus* (Adjanke et al., 2016) and *L. senegalensis* (Omoregie, 2001), it is 30%. However, these discrepancies are expected due to differences between species, in the manufacturing of diets and in experimental conditions. However, studies are needed to enable a product with better nutritional quality. The definition of protocols for fermentation and/or the use of enzymes could be innovative alternatives because these could reduce the deleterious effects of the excess of fibre and other antinutritional factors, which could lead to greater inclusion of these by-products in fish diets (Iluyemi et al., 2010).

5 | CONCLUSIONS

The inclusion of up to 6.26% of PKM (25% corn replacement) in tambaqui diets was efficient in economic terms and did not impair productive performance, health, yield or body mass change, presenting itself as a safe and potential alternative for industrial aquaculture feeds in tropical countries due to its availability, scale production and low cost.

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