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Growth performance, hematological responses and economic indexes of *Colossoma macropomum* (Cuvier, 1818) fed graded levels of glycerol

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ABSTRACT

The aim of this study was to evaluate the performance, hematological responses and economic indicators of juvenile tambaqui *Colossoma macropomum* fed different levels of partial replacement of corn by glycerol (0%, 25%, 50%, 75%, and 100%). The experiment was conducted for 90 days in the production of aquatic organisms lab at the Nilton Lins University, Manaus, Brazil, in a completely randomized design, and consisted of four treatments, four repetitions and two sampling times. In total, 240 juveniles were used with initial average weight and standard length of 15.32 ± 1,61 g and 8.03 ± 0.22 cm, respectively. The fish were maintained in twenty 310 L water tanks that had a closed system with no reuse, continuous aeration, siphoning and replacement of water every 48 h. Feeding was twice a day with the experimental diets, which contained 28% crude protein. The following parameters were considered: zootechnical checks (weight gain + survival %), welfare (health) (hepatosomatic index + condition factor + viscerosomatic index), economic (Economic efficiency rate + economic profitability index) and hematology (hematology + metabolites + ions). The results of the study show that diets for juvenile tambaqui can contain up to 50% replacement of corn by glycerol without compromising the fishes' development under the conditions studied.

1. Introduction

Tambaqui, *Colossoma macropomum* (Cuvier, 1818), is a neotropical freshwater fish with great potential for continental fish farming in Latin America (Aride et al., 2020, 2017; Lima et al., 2020; Nascimento et al., 2020; Pantoja-Lima et al., 2020). In Brazil, it is the second most exploited species in fish farming due to high growth rates, adaptation to intensive farming systems and the quality of its fillet (Rodrigues, 2014; Aride et al., 2017). Despite its importance in Latin American fish farming, there are few studies in the area of nutrition, especially in relation to the replacement of conventional ingredients by alternatives (Oliveira et al., 2006; Aride et al., 2007; Santos et al., 2010; Guimarães and Martins, 2015; Aride et al., 2020).

The most important challenge that the world must face is to feed the increasing population. Humans need regular supply of food. Hence, a healthy diet is necessary to ensure that adequate growing population. Fish represent a good model for healthy sources of protein. Fish production can be increased with use of supplementary feeds (Van Doan

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et al., 2019; Rashidian et al., 2019; Rashidian et al., 2020; Gholampour et al., 2020; Hossein Hoseinifar et al., 2020; Vazirzadeh et al., 2020; Hamed et al., 2021).

Among the alternative ingredients for use in fish nutrition is glycerol, which is a by-product of biodiesel production and can be refined and destined for other purposes, such as, for example, inclusion in animal feed because it is an excellent low-cost source of energy, in addition to being environmentally friendly. Recent studies regarding its use in animal feed have obtained satisfactory zootechnical and physiological results in birds, pigs, ruminants and fish, and indicate that it possible to replace corn and soybean oil at the optimal levels (Cerrate et al., 2006; Balen et al., 2014; Lall and Dumas, 2015; Moesch et al., 2016).

Li et al. (2010) state that diets of up to 10% glycerol for the channel catfish, *Ictalurus punctatus* (Rafinesque, 1818), did not affect fish growth. Neu et al. (2013) observed in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), fry that a level of up to 100% is satisfactory for zootechnical performance and hematological parameters. Gonçalves et al. (2015) suggested a percentage of up to 12% as being the optimal level for juvenile Nile tilapia. Matos et al. (2016) evaluated the zootechnical performance of juvenile tambaqui with diets that replaced corn and soybean oil with crude glycerin and suggested that the level of up to 15% does not affect the growth of animals. However, studies with glycerol in diets that relate growth and physiological conditions have not yet been conducted for tambaqui.

However, when carrying out studies that include alternative ingredients in the fishes' diets, there is also the need to study the hematological aspects of the fish (Burgos-Aceves et al., 2019; Faggio et al., 2015; Faggio et al., 2014a, 2014b, 2014c; Fazio et al., 2013a, 2013b, 2013c).

The variations of blood elements can be evaluated in a qualitative and quantitative way in relation to endogenous and exogenous conditions of organic defense, such as weight, length, nutritional status, intense muscle activity, temperature, pH, concentration of O_2 and CO_2 in the water, making it possible to determine animal welfare (Eckmann, 1987; Tavares-Dias and Sandrim, 1998; Tavares-Dias et al., 1999; Affonso et al., 2002; Aride et al., 2006; De Almeida et al., 2011; Ranzani-Paiva et al., 2005; Moraes and De Almeida, 2014; Fiúza et al., 2015; Araújo et al., 2016; Aride et al., 2016; Chagas et al., 2016; Aride et al., 2017, 2020; Nascimento et al., 2020; Sula et al., 2020).

Thus, the present study aims to evaluate the zootechnical performance, hematological responses and economic indicators of juvenile *C. macropomum* fed diets containing different levels of partial replacement of corn by glycerol.

2. Materials and methods

2.1. Ethical approval

The experiment was approved by the Ethical Review Committee on the Use of Animals at Nilton Lins University (Protocol No.: 015/2015). The study was conducted in accordance with the Brazilian guidelines for animal experiments and was approved by the government of Amazonas state, Brazil. All experiments were conducted according to local and ARRIVE guidelines (Percie du Sert et al., 2020).

2.2. Monitoring of the physical and chemical parameters of the water

During the whole experimental period, temperature (°C), pH, and dissolved oxygen (mg L^{-1}) were measured in the morning (9 am) and afternoon (5 pm), and a total of 180 samples (90 in the morning and 90 in the afternoon) were obtained. Total weekly levels of ammonia (mg L^{-1}) and nitrite (mg L^{-1}) were determined with the aid of 100 mL vials, and samples were analyzed immediately after collection.

All analyses were carried out with the aid of a specific kit (Acquacombo/Técnico – TD 1555, Alfakit, Brazil) following the manufacturer's guidelines. The water quality parameters analyzed showed temperature values of 26.80 ± 0.08 °C; dissolved oxygen, 6.27 ± 0.03 mg L^{-1}; pH, 6.94 ± 0.02; total ammonia, 0.08 ± 0.01 mg L^{-1}; and nitrite, 0.01 ± 0.01 mg L^{-1}.

2.3. Experimental animals, facilities and diets

The study was conducted in the Production of Aquatic Organisms Laboratory (LaPOAq) at the Nilton Lins University, Manaus, Amazonas state (AM) for 100 days (10 days for acclimation and 90 days of experiment). A total of 240 juvenile tambaqui, with a mean initial weight and length of 15.32 ± 1.61 g and 8.03 ± 0.22 cm, respectively, were used. Fish were obtained from the Aquaculture Training, Technology and Production Center (CTTPA), which is located in the district of Balbina, in the Municipality of Presidente Figueiredo, AM, and shipped in plastic bags with oxygen to the LaPOAq. The tambaqui were then randomly distributed in the twenty 310 L circular polyethylene tanks of water (12 per tank), which had a closed system with no recycling, continuous aeration, and water replacement after siphoning that occurred every 48 h throughout the experiment period.

Fish were fed twice a day (9 am and 5 pm) with 4 mm experimental isoenergetic/isoproteic balanced feed, which was elaborated with semi-purified ingredients containing different levels of partial replacement of corn by glycerol (0%, 25%, 50%, 75%, and 100%) for a period of 90 days (Table 1).

The diets were extruded (single thread: 2.5 mm; pellets of around 3 mm) and the animals were fed until the apparent satiety. For extrusion, all ingredients were finely ground in a knife mill (Tecnal®, Piracicaba, Brazil), homogenized and moistened (20% water) at 50 °C. The product was dried in a forced ventilation oven (55 °C) for 24 h, subjected to bromatological analysis (AOAC, 2005) and then stored in a dry place,

Table 1
Composition of experimental feed with different levels of partial replacement of corn by glycerol for juvenile tambaqui, *Colossoma macropomum*.

Ingredients (g/kg)	Glycerol (% in diet)				
	0	25	50	75	100
Soy bran[a]	450.00	450.00	450.00	450.00	450.00
Corn bran[a, b]	300.00	225.00	150.00	75.00	0.00
Wheat bran[b]	135.70	135.50	135.00	137.60	147.00
Fishmeal	58.30	56.50	54.80	52.30	47.70
Glycerol[c]	0.00	75.00	150.00	225.00	300.00
L-lisine[a]	2.20	2.30	2.50	2.70	2.90
DL-methionine[a]	0.10	0.20	0.30	0.30	0.50
Soybean oil[b]	25.80	23.20	20.40	17.20	12.00
Common salt	3.50	8.00	9.00	11.60	11.70
Dicalcium phosphate	20.40	20.30	24.00	24.30	24.20
Premix minerals and vitamins[d]	4.00	4.00	4.00	4.00	4.00
Nutrients[e]					
Crude protein (g/kg)[a]	280.00	280.00	280.00	280.00	280.00
Digestible energy (MJ/kg)[a,c]	13.807	13.807	13.799	13.807	13.807
Calcium (g/kg)	10.50	10.50	10.50	10.50	10.50
Phosphate (g/kg)	7.00	7.00	7.00	7.00	7.00
Cost of the diet (R $/kg)[f]	1.31	1.27	1. 22	1.17	1.13

[a] According to Rodrigues (2014).
[b] Digestibility rate according to Guimarães et al. (2014).
[c] Digestibility rate according to Balen et al. (2014).
[d] Mineral and vitamin micronutrient levels (Premix Nutrifish Guabi®, São Paulo, Brazil) per kg^{-1} of product: Fe 15,000 mg; Cu 2500 mg; Zn 12,500 mg; I 375 mg; Mn 12,500 mg; Se 87.5 mg; Co 125 mg; vitamin A 2,500,000 IU; vitamin D3 600,000 IU; vitamin E 37,500 IU; vitamin K 3750 mg; vitamin C 50,000; vitamin B1 4000 mg; vitamin B2 4000 mg; vitamin B6 4000 mg; vitamin B12 4000 µg; pantothenic acid 12,000 mg; biotin 15 mg; folic acid 1250 mg; niacin 22,500 mg; BHT 15,000 mg.
[e] Nutrient levels were calculated using the software Super Crac Premium®.
[f] Considering the exchange rate on 02/03/2021 of US $ 1.00 = R$ 5.37.

with a temperature between 20 and 24 °C and at 1 m from the ground.

2.4. Zootechnical performance and experimental design

A completely randomized design (CRD) was used, which consisted of four treatments and one control (0%), with four repetitions. Twelve fish were sampled per treatment at two sample times (baseline = 6 fish, and at 90 days = 6 fish = 12 fish per treatment). Before the biometric evaluation, the supply of feed was suspended for 48 h and, during biometrics, the animals were sedated for 3 min with Eugenol® at a dose of 35 mg L^{-1} (Roubach et al., 2005). Subsequently the animals were weighed and measured to estimate the growth parameters.

At each sample time, eight fish per treatment were euthanized and dissected for the removal of the livers and viscera in order to determine the hepatosomatic index (Bombardelli et al., 2010) and viscerosomatic index (Ng and Wang, 2011). The carcasses, muscles and digestive tracts were immediately frozen (−80 °C) for use in subsequent analyses. The following parameters were analyzed: weight gain (WG; g, wet weight), feed consumed (FC; g, dry weight), apparent food conversion (AFC), specific growth rate (SGR, %), protein efficiency rate (PER, %), condition factor (CF), Survival (S, %), hepatosomatic index (HSI; %) and viscerosomatic index (VSI; %).

The growth performance was verified using the following equations:

- Weight gain, WG (%) = 100 × [(final weight (g) − initial weight (g)) / initial weight (g)];
- Feed consumed, FC (%) = [dry feed intake (g) / final fish weight (g) / days fed] × 100;
- Apparent food conversion, AFC (kg/kg) = dry feed fed (kg) / body weight gain (kg);
- Specific growth rate, SGR (%) = 100 * (Natural logarithm of final average weight − natural logarithm of the initial mean weight) ÷ experiment days
- Protein efficiency rate, PER (%) = [body weight gain (g) / protein intake (g)] × 100;
- Condition factor (CF) = 100 * weight(g) ÷ Standard length $(cm)^3$;
- Survival (S, %) = 100 * Number of final fish per treatment ÷ Initial number of fish per treatment;
- Hepatosomatic index, HSI (%) = [liver weight (g) / fish weight (g)] × 100;
- Visceral fat index, VFI (%) = [visceral fat weight (g) / fish weight (g)] × 100;

2.5. Hematological parameters

A factorial CRD was used, which consisting of four treatments and a control, four repetitions, with four fish per treatment at two collection times (baseline and 90 days). For the evaluation of the hematological parameters, 1.0 mL of blood was taken by caudal puncture. The circulating erythrocyte count (RBC) was determined using a Neubauer chamber after dilution of whole blood in formalin-citrate (Oliveira et al., 2016), with reading under a microscope (Leica, DM3000, Germany). Hemoglobin concentration (Hb) was determined by the cyanmethemoglobin method and the hematocrit by the microhematocrit method. Subsequently, the hematimetric indices were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using formulas described from the RBC, Hb and Ht.

The plasma was obtained after centrifugation at 750 rpm for 10 min and immediately frozen (−20 °C) for later use in the biochemical analysis. The biochemical blood variables included the quantification of glucose, triglycerides, cholesterol, total proteins, albumin, calcium, urea and phosphorus levels, which were determined with the aid of specific commercial laboratory kits (Labtest Diagnostica S. A., Brazil) for each parameter, with subsequent reading on a spectrophotometer (Multiskan™, Thermo Fisher Scientific, Brazil).

Plasma ions sodium (Na^+) and potassium (K^+) were analyzed using flame photometry (DM-62, Digimed, Brazil), chloride (Cl^{-1}) and ammonia ($NH4^+$) levels were determined with the aid of laboratory kits (Labtest Diagnostica S. A., Brazil) and spectrophotometer reading at specific wavelengths for each assay, according to the manufacturer's instructions.

2.6. Economic indicators

The price of the ingredients that are used in the calculation of the cost of the diet was obtained from Biomercado© (Viçosa, Minas Gerais state, Brazil, based on the average national exchange rate of US$ 1 = 5.37 Brazilian Reais (R$) on Jan 3th, 2021): soybean bran: R$ 0.80 kg; corn: R$ 1.28 kg; wheat bran: R$ 1.15 kg; fishmeal: R$ 1.65 kg; glycerol: R$ 0.70 kg; soybean oil: R$ 1.28 kg common salt: R$ 0.15 kg; Premix vitamins & minerals: R$ 1.28 kg; Dicalcium phosphate: R$ 1.02 kg; L-Lysine: R$ 2.56 kg, DL-Methionine: R$ 2.24 kg. The cost of processing (R$ 0.20 kg), bagging (R$ 0.20 bag) and logistics were included in the price of the diets. The logistics used was from Ribeirão Preto, SP to Manaus, AM at a cost of R$ 4400.00 per ton.

Tambaqui selling price (TSP per kg) = fry cost (R$ per individual) ÷ mean final weight (MFW, g) × 1000. We used the cost of R$ 0.47 per juvenile tambaqui, divided by the final experimental mean weight of 58.28 g multiplied by 1000 (convert to kg) i.e., (0.47 ÷ 58.28 × 1000) = 8.06. Therefore, the TSP was estimated at R$ 8.00 per kg. The indicators were evaluated according to Martínez-Llorens et al. (2007):

Economic efficiency rate (EER, R$ per kg) (modified) = diet offered ÷ final number of fish per treatment (kg) × diet cost (kg) ÷ WG (kg); economic profitability index (EPI, R$ fish) = EER (kg of fish) × WG (kg) − MFW (kg of fish) × TSP.

2.7. Statistical analysis

The results were expressed as mean ± standard deviation. The Shapiro-Wilk test was used to evaluate normality and the Bartlett test to evaluate the homoscedasticity of the data. When the distribution was normal, analysis of variance (ANOVA) was applied. Nonparametric data were evaluated using the Kruskal-Wallis test. For parametric ANOVA, the Tukey test was applied when there was a difference between treatments and this was graphically represented by quadratic regression. The 5% level of significance was considered for all analyses (Zar, 2010; Volpato and Barreto, 2016).

The data were analyzed using the R (version 3.4) software package (Rcmdr) plug-in (FactoMineR). Principal component analysis (PCA) was applied to better represent the interactions between the variables studied considering the following factors: treatments (substitution levels), time (baseline and final), weight and length. Only the first two components of the PCA were used for interpretation.

3. Results

3.1. Zootechnical performance and body indices

A quadratic effect ($P < 0.05$) was observed on weight gain and protein efficiency rate (Figs. 1 and 2). With the derivation of the equations of weight gain $y = -0.0027x^2 + 0.201x + 42.288$ and protein efficiency rate $y = -0.0096x^2 + 0.7179x + 151.03$, it appears that 50.0% of corn substitution by glycerol offers better results for weight gain (47.99 g) and protein efficiency rate (171.38%) for juvenile tambaqui (Table 2).

The specific growth rate increased by 50% of the glycerol level, the apparent food conversion varied between 0.48 and 0.62, the hepatosomatic index increased by 75% glycerol and viscerosomatic index increased by 75% and 100% glycerol (Table 2).

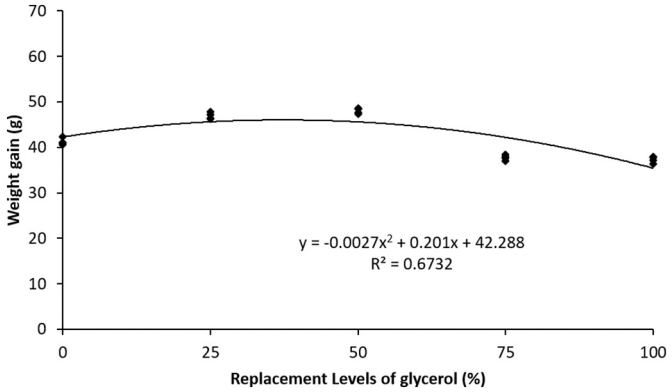

Fig. 1. Weight gain (g) of juvenile tambaqui fed with different levels of partial replacement of corn by glycerol.

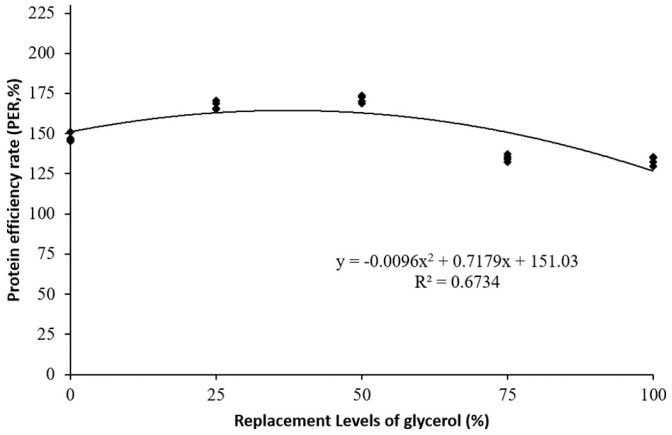

Fig. 2. Protein efficiency rate (%) of juvenile tambaqui fed with different levels of partial replacement of corn by glycerol.

3.2. Hematology

The observed results of the erythrogram, metabolites and ions are expressed in Tables 3, 4 and 5, respectively. For the 25% level of glycerol, an increase in hematocrit and RBC values, a reduction in MCHC and no difference in hemoglobin concentration and MCH were portrayed (Table 3).

For the metabolites, when 50% of the glycerol level was added, similarity was observed without the level of glucose, cholesterol, total proteins and urea (Table 4). For the ions, the similarity in the values of Na^{+}, K^{+}, Ca^{2+}, NH^{4+} and Cl^{-1} was also portrayed (Table 5). Interactions between the studied variables were graphically represented by the principal component analysis (PCA) (Figs. 3 and 4).

Significant interaction was observed (X = 99.84; Y = 0.16; X + Y = 100) in the properties regarding the erythrogram (Fig. 3) where there was strong interaction on the X axis, demonstrating clear distinction between the baseline (left side = negative) and final (right side = positive) and Y axis (upper = positive; lower = negative) when compared the experimental conditions involving the feed with added glycerol (0%, 25%, 50%, 75%, 100%).

3.3. Economic indicators

In regards to the corn, it can be observed that the cost of production tends to reduce in accordance with the increases in the level of glycerol substitution in the diet, as shown in Table 1.

4. Discussion

4.1. Zootechnical performance

The replacement of 50% of corn by glycerol offers tambaqui weight gain and improvements in protein efficiency rate (Table 2). In studies in which glycerol was used as an energy source in the feeding of fish, such as Nile tilapia, carp, *Cyprinus carpio* (Linnaeus, 1758), channel catfish and tambaqui, satisfactory results were presented for growth and proved it to be a viable option for replacing corn and soybean oil in the nutrition of these fish (Graeff and Tomazelli, 2007; Li et al., 2010; Meurer et al., 2012; Neu et al., 2012, 2013; Matos et al., 2016; Moesch et al., 2016).

Values obtained in the present study indicate that there is a tendency to decrease growth after the level of 50.0% glycerol substitution.

Table 2
Mean values of zootechnical performance of juvenile tambaqui (*Colossoma macropomum*) with different levels of partial replacement of corn by glycerol for the period of 90 days.

Parameters[a]	Glycerol levels (% in diet)					p^{b}	p^{c}	CV[d]
	0	25	50	75	100			
Final weight (g)	56.82 ± 0.41[b]	64.00 ± 0.38[a]	64.44 ± 0.35[a]	53.44 ± 0.55[c]	52.73 ± 0.41[d]	0.41	0.95	0.73
Final standard length (cm)	12.18 ± 0.17[c]	12.54 ± 0.27[b]	12.88 ± 0.24[a]	11.85 ± 0.12[d]	11.85 ± 0.09[d]	0.39	0.43	1.55
Weight gain (g)[e]	41.25 ± 0.75[c]	46.91 ± 0.70[b]	47.99 ± 0.62[a]	37.74 ± 0.58[d]	37.27 ± 0.75[d]	0.35	0.99	1.62
Specific growth rate (%, day)[e]	1.44 ± 0.04[b]	1.47 ± 0.03[b]	1.52 ± 0.03[a]	1.36 ± 0.02[c]	1.36 ± 0.04[c]	0.07	0.90	2.11
Protein efficiency rate (%)[e]	147.31 ± 2.67[c]	167.55 ± 2.50[b]	171.38 ± 2.20[a]	134.78 ± 2.09[d]	133.12 ± 2.69[d]	0.35	0.99	1.63
Apparent food conversion	0.51	0.50	0.48	0.61	0.62	–	–	–
Survival (%)	93.75	91.67	95.83	87.50	85.42	–	–	–
Body índices								
Hepatosomatic index (%)[e]	1.58 ± 0.25[c]	1.67 ± 0.22[b,c]	1.66 ± 0.15[b,c]	2.18 ± 0.11[a]	1.95 ± 0.24[a,b]	0.46	0.68	11.08
Viscerosomatic index (%)[e]	11.57 ± 0.86[b,c]	11.13 ± 0.14[c]	11.33 ± 0.39[c]	12.90 ± 1.01[a]	12.52 ± 0.86[a,b]	0.99	0.06	6.17
Condition fator	3.15 ± 0.11	3.25 ± 0.22	3.02 ± 0.16	3.21 ± 0.09	3.17 ± 0.06	0.57	0.32	4.45
Economic indicators								
Economic efficiency rate (R$ kg)	0.67	0.63	0.59	0.72	0.69	–	–	–
Economic profitability index (R$ $fish^{-1}$)	0.43	0.48	0.49	0.40	0.40	–	–	–

[a] Data expressed in means and ± standard deviation ($N = 12$). Values on the same line with equal letters do not differ from each other by the Tukey test ($p > 0.05$).
[b] According to the Shapiro-Wilk test ($p < 0.05$) the data are considered normal.
[c] According to the Bartlett test ($p < 0.05$) the data are considered homogeneous.
[d] Coefficient of variation (%).
[e] Data were graphically represented by quadratic regressions.

Table 3
Mean values of erythrogram of juvenile tambaqui (*Colossoma macropomum*) fed diets containing different levels of partial replacement of corn by glycerol for the period of 90 days.

Parameters	Crude glycerol levels (%)													
	0		25		50		75		100		p[b,c]		CV (%)[d]	
	0	90	0	90	0	90	0	90	0	90	0	90	0	90
Hematocrit (Ht, %)	27.5 ± 2.1	26.5 ± 1.3[c]	24.0 ± 1.4	29.3 ± 0.9[a]	24.7 ± 1.0	28.0 ± 0.8[a,b]	25.0 ± 1.2	28.5 ± 0.6[a,b]	25.5 ± 1.7	27.5 ± 0.6[b,c]	–	–	6.0	3.2
Hemoglobin concentration (Hb, g/dL)	1.4 ± 0.1	3.1 ± 0.1[a]	1.3 ± 0.1	3.1 ± 0.1[a]	1.4 ± 0.2	3.1 ± 0.1[a]	1.4 ± 0.1	3.1 ± 0.1[a]	1.5 ± 0.1	2.3 ± 0.1[b]	–	–	8.4	3.0
Circulating erythrocyte count (RBC, million/μL)	1.9 ± 0.2[b]	2.3 ± 0.1[b]	1.8 ± 0.1[b]	2.6 ± 0.2[a]	2.2 ± 0.1[a]	2.6 ± 0.2[a]	1.9 ± 0.1[b]	2.1 ± 0.1[b]	2.4 ± 0.1[a]	2.6 ± 0.2[a]	A*	–	5.3	5.4
Mean corpuscular volume (MCV, fL)	140.8 ± 2.8[a]	114.1 ± 2.0[b]	130.6 ± 6.3[b]	112.6 ± 5.3[b,c]	111.5 ± 4.3[c]	107.4 ± 4.4[c,d]	129.6 ± 2.8[b]	134.1 ± 4.6[a]	107.4 ± 4.9[c]	104.5 ± 0.5[d]	–	–	3.6	3.4
Mean corpuscular hemoglobin concentration (MCHC, %)	5.1 ± 0.6	11.6 ± 0.6[a]	5.6 ± 0.9	10.5 ± 0.5[b]	5.7 ± 0.4	10.9 ± 0.3[b]	5.7 ± 0.5	10.8 ± 0.2[b]	6.0 ± 0.1	8.5 ± 0.2[c]	–	–	10.0	3.9
Mean corpuscular hemoglobin (MCH, g/dL)	7.2 ± 0.8	13.2 ± 0.8[a]	7.3 ± 0.8	11.8 ± 0.9[a]	6.3 ± 0.7	11.7 ± 0.6[a]	7.3 ± 0.5	14.5 ± 0.6[a]	6.5 ± 0.3	4.4 ± 4.9[b]	–	A*	9.4[e]	20.8

[a] Data expressed in means and ± standard deviation, ($N = 4$).
[b] According to the Shapiro-Wilk test ($p < 0.05$), residues with lowercase letters (a) cannot be considered normal.
[c] According to the Bartlett test ($p < 0.05$), residues with capital letters (A), cannot be considered homogeneous.
[d] Coefficient of variation.
[e] Values on the same line with equal letters do not differ from each other by the Tukey test ($p > 0.05$).
[*] According to the Kruskal-Wallis test ($p < 0.05$), the data are considered non-parametric.

Table 4
Mean values of metabolites of juvenile tambaqui (*Colossoma macropomum*) fed diets containing different levels of partial replacement of corn by glycerol for the period of 90 days.

Parameters	Crude glycerol levels (%)													
	0		25		50		75		100		p[b,c]		CV (%)[d]	
	0	90	0	90	0	90	0	90	0	90	0	90	0	90
Glucose (mg/dL)	38.0 ± 5.1[c]	72.6 ± 0.2[c]	53.1 ± 3.1[b]	73.2 ± 0.3[a,b]	62.5 ± 1.8[a]	73.0 ± 0.2[b]	64.7 ± 3.6[a]	73.4 ± 0.2[a]	17.9 ± 3.6[d]	71.8 ± 0.3[d]	–	–	7.7	0.3
Cholesterol (mg/dL)	86.5 ± 1.3[a]	110.3 ± 0.5[c]	61.0 ± 1.8[b]	112.6 ± 0.2[a,b]	59.2 ± 0.4[b,c]	112.7 ± 0.3[a]	58.9 ± 0.4[c]	112.2 ± 0.2[b]	58.0 ± 1.6[c]	112.5 ± 2.3[a,b]	–	–	2.0	0.3
Triglycerides (mM/L)	81.8 ± 5.0[c]	125.8 ± 1.4[c]	98.5 ± 4.9[b]	110.8 ± 2.5[d]	120.7 ± 1.7[a]	110.9 ± 5.3[d]	117.0 ± 6.6[a]	136.1 ± 4.8[b]	75.9 ± 5.7[c]	145.9 ± 3.5[a]	–	–	5.1[e]	3.0
Total proteins (g/dL)	2.9 ± 0.1[a]	3.0 ± 0.1	2.4 ± 0.1[c]	3.0 ± 0.1	2.6 ± 0.1[b]	3.0 ± 0.1	2.9 ± 0.1[a]	3.0 ± 0.1	2.9 ± 0.1[a]	3.0 ± 0.1	a*	–	2.1	0.5
Albumin (g/dL)	0.8 ± 0.1[c]	1.1 ± 0.1[c]	0.7 ± 0.1[b]	1.3 ± 0.1[a]	0.8 ± 0.1[b]	1.4 ± 0.1[a]	0.9 ± 0.1[a]	1.21 ± 0.1[b]	0.9 ± 0.1[a]	1.3 ± 0.1[a]	–	aA*	2.0	3.2
Phosphorus (mg/dL)	8.9 ± 0.1[c]	13.6 ± 0.2[a]	9.1 ± 0.2[c]	11.4 ± 0.1[b]	12.3 ± 0.4[b]	10.8 ± 0.1[c]	12.7 ± 0.3[a,b]	10.8 ± 0.2[c]	13.0 ± 0.5[a]	13.6 ± 0.4[a]	–	–	3.1	1.8
Urea (mg/dL)	181.9 ± 2.0[b]	157.5 ± 3.7[b]	201.9 ± 7.0[a]	157.2 ± 2.8[b]	201.5 ± 6.2[a]	157.5 ± 0.7[b]	183.9 ± 4.3[b]	157.6 ± 2.0[b]	154.3 ± 3.1[c]	168.1 ± 3.1[a]	–	–	2.9	1.7

[a] Data expressed in means and ± standard deviation, ($N = 4$).
[b] According to the Shapiro-Wilk test ($p < 0.05$), residues with lowercase letters (a) cannot be considered normal.
[c] According to the Bartlett test ($p < 0.05$), residues with capital letters (A), cannot be considered homogeneous.
[d] Coefficient of variation.
[e] Values on the same line with equal letters do not differ from each other by the Tukey test ($p > 0.05$).
[*] According to the Kruskal-Wallis test ($p < 0.05$), the data are considered non-parametric.

Therefore our results differ from the results presented by Matos et al. (2016) who observed that up to the level of 15.0% substitution in diets for juveniles tambaqui there was no difference in growth. The present study differed from the studies of Neu et al. (2012), Neu et al. (2013), and Graeff and Tomazelli (2007) who described that there was no significant difference in zootechnical performance, and suggested the substitution of up to 100.0% corn by crude glycerol for juvenile Nile tilapia and juvenile carp. Li et al. (2010) observed in diets for channel catfish that the use of up to 10.0% crude glycerol did not influence growth or physiological conditions.

For the hepatosomatic and viscerosomatic index (Table 2), there was an increasing trend, according to the increase in glycerol levels, and thus it can interfere with energy metabolism, accelerating or increasing the deposition of visceral fat and energy in the form of glycogen and/or lipids in the liver (Moesch et al., 2016). As such, the results of the present study do not agree with those of Matos et al. (2016) in which they observed that the hepatosomatic index showed no alterations up to the level of 15% glycerol substitution, however the authors did not test the maximum level of substitution. Accordingly, the food conversion indicated that glycerol satisfies the energy needs of the fish under the studied conditions, replacing corn satisfactorily, but with a tendency to increase in AFC after the level of 50.0% substitution.

Table 5
Mean values of ions of juvenile tambaqui (*Colossoma macropomum*) fed diets containing different levels of partial replacement of corn by glycerol for the period of 90 days.

Parameters	Crude glycerol levels (%)													
	0		25		50		75		100		$p^{b,c}$		CV (%)[d]	
	0	90	0	90	0	90	0	90	0	90	0	90	0	90
Na^{+}	191.3 ± 2.9[a]	162.0 ± 0.8	181.0 ± 7.0[b]	162.8 ± 1.3	160.0 ± 6.2[d]	163.0 ± 1.8	153.0 ± 2.2[d]	164.3 ± 1.0	170.5 ± 5.2[c]	163.5 ± 1.3	–	–	2.9	0.8
K^{+}	6.2 ± 0.2[b]	3.8 ± 0.2	6.4 ± 0.2[b]	3.5 ± 0.2	6.6 ± 0.3[a,b]	3.7 ± 0.3	6.7 ± 0.1[a,b]	3.8 ± 0.1	7.2 ± 0.5[a]	3.8 ± 0.2	A	aA*	5.5	5.1
Ca^{2+}	5.0 ± 0.2[a]	6.7 ± 0.3	5.0 ± 0.3[a]	6.6 ± 0.4	3.4 ± 0.5[c]	5.6 ± 0.4	3.8 ± 0.5[b]	6.9 ± 0.3	4.2 ± 0.1[b]	7.2 ± 0.1	–	–	6.1	4.7
NH^{4+}	0.06 ± 0.01[a]	0.14 ± 0.01[a]	0.06 ± 0.01[a]	0.08 ± 0.01[c]	0.05 ± 0.01[b]	0.07 ± 0.01[d]	0.05 ± 0.01[b]	0.09 ± 0.01[b]	0.03 ± 0.01[c]	0.08 ± 0.01[c]	aA*	aA*	7.8[e]	5.2
Cl^{-1}	107.9 ± 2.1[d]	107.9 ± 0.2[b]	131.9 ± 1.7[a]	107.6 ± 0.2[c]	127.9 ± 0.6[b]	107.6 ± 0.1[c]	123.1 ± 1.2[c]	108.3 ± 0.1[a]	95.0 ± 3.4[c]	108.6 ± 0.2[a]	–	–	1.7	0.2

[a] Data expressed in means and ± standard deviation, ($N = 4$).
[b] According to the Shapiro-Wilk test ($p < 0.05$), residues with lowercase letters (a) cannot be considered normal.
[c] According to the Bartlett test ($p < 0.05$), residues with capital letters (A), cannot be considered homogeneous.
[d] Coefficient of variation.
[e] Values on the same line with equal letters do not differ from each other by the Tukey test ($p > 0.05$).
[*] According to the Kruskal-Wallis test ($p < 0.05$), the data are considered non-parametric.

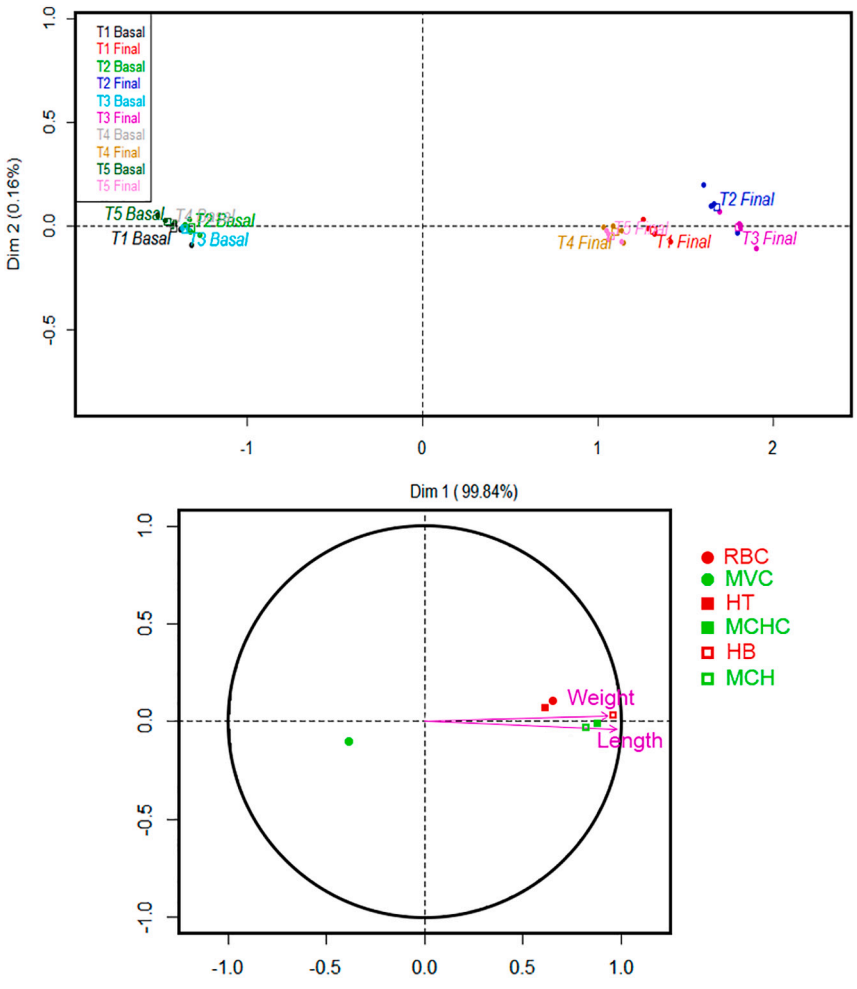

Fig. 3. A, B. Principal component analysis (PCA) of erythrogram of juvenile tambaqui, *Colossoma macropomum* fed diets containing different levels of partial replacement of corn by glycerol.
Legend: (A) T1 Baseline color (black) = level 0%; T2 Baseline color (light green) = level 25%; T3 Baseline color (sky blue) = level 50%; T4 Baseline color (gray) = level 75%; T5 Baseline color (dark green) = level 100%; T1 Final color (red) = level 0%; T2 Final color (dark blue) = level 25%; T3 Final color (dark pink) = level 50%; T4 Baseline color (ochre) = level 75%; T5 Baseline color (light pink) = level 100%.

4.2. Hematology

The hematological alterations observed in the present study are associated with the growth of animals as a result of the time, since, as described by Tavares-Dias and Sandrim (1998) the increase in the variables related to the erythrogram are proportional to the increase in body mass and total length of the tambaqui.

In the present study, from the results described in Fig. 3B, we demonstrate that in the treatments there is strong positive interaction with weight at the substitution levels of 25% and 50% when correlated

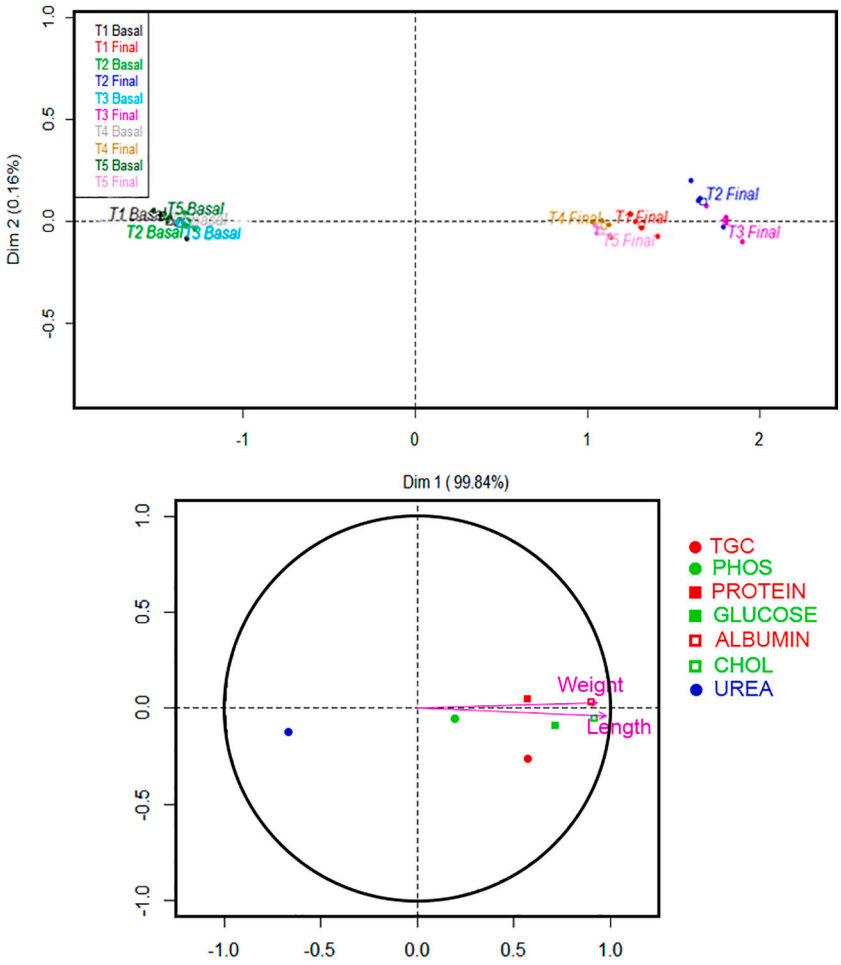

Fig. 4. A, B. Principal component analysis (PCA) of plasma metabolites of juvenile tambaqui, *Colossoma macropomum* fed diets containing different levels of partial replacement of corn by glycerol.
Legend: (A) T1 Baseline color (black) = level 0%; T2 Baseline color (light green) = level 25%; T3 Baseline color (sky blue) = level 50%; T4 Baseline color (gray) = level 75%; T5 Baseline color (dark green) = level 100%; T1 Final color (red) = level 0%; T2 Final color (dark blue) = level 25%; T3 Final color (dark pink) = level 50%; T4 Baseline color (ochre) = level 75%; T5 Baseline color (light pink) = level 100%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to hematological parameters (RBC, Ht and Hb), in addition to positive interaction with length (X axis) with a negative tendency in the Y axis in the corpuscular constants (MCHC and MCH) in 75% and 100%, respectively, indicating that the glycerol level has increased may allow tambaqui to require less hemoglobin at the cellular level. This is similar to what Li et al. (2010) observed for the channel catfish, obtaining a positive trend with a level of 10% for Hb related to weight and length. The baseline clearly showed a grouping between the treatments for all variables and indicated a tendency of homeostasis and confirmed that all animals had the same initial conditions. Oliveira et al. (2017), in studies with three species of freshwater stingrays, and Castro et al. (2020), in studies with three peacock bass species also observed interactions between the parameters of the erythrogram.

The interactions observed in relation to plasma metabolites are represented in Fig. 4, for which strong interactions occurred (X = 99.84; Y = 0.16; X + Y = 100). Thus, it is demonstrated by the Y axis (25% had interaction with a positive tendency and 50% had a negative tendency) that both presented interactions superior to the treatments (0%, 75% and 100%) (Fig. 4B). Interaction was also observed with a positive trend with weight in the (X + Y axes) final time and treatments, for total proteins and albumin, justified by the increase in the animal's weight over the 90 days, and interaction with a negative trend with respect to length in levels of glucose, phosphorus, triglycerides and cholesterol between the different levels of substitution of corn by glycerol, which indicates that animals are not converting energy into fat.

Of these metabolites, the triglycerides (TGC) can be highlighted (Table 3), since the higher the substitution level, the higher their plasma levels were. Triglycerides and phospholipids make up the class of lipids by structuring the cell membranes in the lipid bilayer and with immersed proteins. Glycerol in the metabolism, according to Min et al. (2008), plays a structural role for triglycerides and phospholipids by providing energy in the glycolytic and carboxylic acid pathways. Therefore it is osmoregulatory, i.e., it aims to restore and maintain the normal volume of pressure and the activity of cells (Lin, 1977).

4.3. Economic indicators

The cost of ingredients for the manufacture of feed is high for the fish feed manufacturing industry, especially for those located in the Amazon region due to the difficulty of transport in the region. In relation to corn, it can be observed that the cost of feed production tends to decrease with the increase in the level of substitution by glycerol in the diet (Table 1), thus, in fish farming, the cost of feed can correspond to 70% of production costs (Bicudo et al., 2010).

In this context, the analysis of the economic indicators applied in the experiments with alternative diets allows us to estimate the success with the adoption of new ingredients. Thus, in this study the economic efficiency rate (EER) undergoes changes related to apparent food conversion (AFC); the economic profitability index (EPI) with weight gain (WG); specific growth rate (SGR) and protein efficiency rate (PER) (Table 2). Despite the 100% substitution level presenting the lowest feed cost, it was not the best solution, since the final biomass is influenced by survival, which in turn caused a reduction in profits. Among the two economic indices evaluated, the one that best represented the responses of zootechnical performance was the EPI, which followed the variations between the substitution levels for these zootechnical data and confirmed that the levels of 25% and 50% responded better economically.

Sweilum et al. (2005) evaluated the economic returns of juvenile Nile tilapia and reportied that there was an inversely proportional relationship between crude protein (CP) and digestible energy (DE), when CP was elevated in diets, the economic returns increased, but the economic returns reduced when the level of DE was elevated in diets. This suggests the intermediate level of this relationship between CP and DE. Bicudo et al. (2010) analyzed the same economic indicators for juvenile pacu, *Piaractus mesopotamicus* (Holmberg, 1887), using diets containing different levels of protein and digestible energy found that the EER was proportional to the level of protein and digestible energy in the diet.

Thus, the present study demonstrates that the effect of economic indicators on diets formulated using fixed values of DE/CP (isoenergetic and isoproteic) and replacing a conventional ingredient with alternatives, and highlights the participation of the tested ingredient. According to Martínez-Llorens et al. (2007), these economic indicators consider important technical effects and may be common tools in nutritional studies for fish. It is important to consider that the results obtained for the EPI were calculated for the weight of whole fish with viscera, and did not consider the yields of carcass and fillet separately. This would further increase the accuracy of the evaluation, which is fundamental for the nutrition of fish and the final price of the product.

5. Conclusion

The levels of 25% and 50% glycerol provided the best results in the phase and conditions studied. Considering the zootechnical parameters (WG + S%), well-being (healthiness) (VSI + HSI + CF), economic (EER + EPI) and hematological (erythrogram + metabolites + ions), the diets for juvenile tambaqui can receive up to 50% replacement of corn by glycerol without compromising the species.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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