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Performance and physiological response of juvenile tambaqui (*Colossoma Macropomum*, Curvier 1818) feeding with pequi oil (*Caryocar Brasiliense*, Camb)

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ABSTRACT. The aim of the study was to evaluate the performance and physiological responses of juvenile tambaqui (Colossoma macropomum) fed diets containing pequi oil (Caryocar brasiliense, Camb). A total of 240 juveniles were observed, with an average weight of 17.91±4.87g, distributed in 20 boxes with a capacity of 500 liters each, in a completely randomized design with five treatments and four replications, containing 12 fish per replication. Five isoprotein diets (41% of crude protein) containing increasing levels of pequi oil (0.5, 1.5, 2.0, 3.8 and 5.5%) were assessed. The animals were kept in a water recirculation system, equipped with a filter, forced aeration, and a 12-hour photoperiod. To evaluate the performance, survival rate, weight gain, feed conversion, specific growth rate, protein efficiency rate, hepatosomatic index, and digestive-somatic index were analyzed. For physiological responses, hematological parameters and indexes, and differential leukocyte counts were determined. The concentration of serum protein, albumin, and globulin was also determined at the end of a 60-day period. There was no effect of the inclusion of pequi oil on weight gain, feed conversion, and protein efficiency rate ($p > 0.05$). The specific growth rate was significantly different ($p < 0.05$) with the reduction of treatments. An increase in the hepatosomatic index was observed in the treatments, with significant differences ($p < 0.05$) and a linear increase, resulting in evidence of a lack of essential fatty acids. Hematocrit values showed no differences, but discrepancies were observed ($p < 0.05$) for the number of erythrocytes, which, however, remained within the reference values for the species. The hematometric ratios also showed differences between treatments ($p < 0.05$), remaining within the reference values, not characterizing an anemic condition in the animals. The increase in serum protein levels suggests that pequi oil starts to act as an immunostimulant. However, the use of pequi oil in juvenile tambaqui was not able to guarantee an efficient growth rate.

Keywords: hematology; zootechnical performance; vegetal oil; firm farming.

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Introduction

Brazilian aquaculture has shown growth in recent years, with emphasis on the production of tambaqui (Colossoma macropomum, CURVIER 1818). The species is the second most produced by Brazilian fish farms, due to its high growth rate and low production cost compared to others, in addition to being the main source of protein for low-income communities in the North of Brazil (Guimarães & Martins, 2015).

The aquaculture sector uses 80% of global fish oil production, which is the main source of lipids in the diet of fish species (National Research Council [NRC], 2011; Demir, Türker, Acar, & Kesbiç, 2014). As fish oil prices vary depending on the fishing industry and with the growth of aquaculture, fish oil production will not be able to meet the demand (Yildirim, Acar, Türker, Sunar, & Yilmaz, 2013).

An alternative to limited access to this essential ingredient has been to replace fish oil with vegetable oils (Naylor et al., 2009; Kenari, Mozanzadeh, & Pourgholam, 2011). This alternative source can improve the use of ingested protein, reduce the cost of feeding, and provide essential fatty acids necessary for development (Martino, Cyrino, Portz, & Trugo, 2002), and promote better health in fish (Montero et al., 2003; 2008, Kiron, Paungkaew, Ishizaka, Satoh, & Watanabe, 2004; Mourente, Good, Thompson, & Bell, 2007).

Freshwater fish have an innate ability to elongate and desaturate linoleic acid (LA) and linoleic acid LNA into eicosapentaenoic acid (EPA), arachidonic acid (ARA), and docosahexaenoic acid (Sargent, Tocher, & Bell, 2002). That said, the imbalance between EFAs promotes competition between enzyme sites. However, the introduction of diets rich in n-6 PUFA can alter the n-3/n-6 ratio influencing the composition of the immune system, including leukocytes (Thompson et al., 1996; Mourente, Good, & Bell, 2005).

Total or partial replacement of fish oil with vegetable oils in fish has been previously suggested (Ng & Low, 2005; Turchini, Torstensen, & Ng, 2009). The use of vegetable sources of lipid, such as corn oil (Martino et al., 2002), soybean oil (Reis et al., 1989), sunflower oil (Hoffman & Prinsloo, 1995), canola (Bibiano et al., 2002), and linseed (Vargas, Souza, Kessler, & Baggio, 2008; Yildiz, Köse, Issa, & Kahraman, 2015), was tested on fish.

The pequi tree (Caryocar brasiliense Camb.) has great economic relevance, given that its fruit, the pequi, is characterized by the presence of bioactive compounds and many nutrients, especially in the mesocarp (Vieira & Martins, 2000; Bailão, Devilla, Conceição, & Borges, 2015).

The fruit has a high content of lipids and carotenoids in its' pulp (Lima, Silva, Trindade, Torres, & Mancini-Filho, 2007).The objective of this study was to investigate the effect of adding pequi (Caryocar brasiliense Camb.) pulp oil on the zootechnical performance and physiological responses (hematological parameters and immune response) of juvenile tambaqui (Colossoma macropomum, CURVIER 1818).

Material and methods

Test species

A total of 240 juvenile tambaqui with an average weight of 17.91±4.87 g and an average total length of 9.38±0.90 cm (Colossoma macropomum) were used. The animals used in the experiment were collected in commercial fish farms (Brejinho de Nazaré, Tocantins, Brazil, latitude 11º 00' 00'' south; longitude 48º 33' 56'' west) and then transported to the aquaculture laboratory of the Federal Institute of Education, Science and Technology of Tocantins – Palmas Campus, to perform initial biometrics, recovery from transport stress, and acclimatization to laboratory conditions. During this period and throughout the experiment, the animals were kept in an enclosed water recirculation system equipped with a lake filter and forced aeration (air blower), with the photoperiod adjusted to 12 hours. The experiment was carried out in a completely randomized design, with 5 treatments (inclusion levels) and 4 replications (boxes). The experimental procedures were approved by the Animal Ethics Committee of the University of Brasilia (UnBDoc 66730/2016).

Experimental diets

Five isoprotein diets (41% of crude protein), with 5 different levels of pequi oil inclusion (0.5, 1.5, 2.0, 3.8, and 5.5%) were fed to the animals (Table 1) with the supply of 4100 Kcal kg^{-1} Gross energy (Van der Meer, 1997) as reference. The ingredients were ground and sieved (Ø=0.7 ± 0.2mm), uniformly homogenized, mixed with the oil, and then pelleted in the Aquaculture laboratory at IFTO-Palmas. The rations were kept in cooled plastic bags (4ºC).

Table 1. Centesimal composition of experimental diets.

	E.B Kcal kg^{-1}				
Ingredients	3900	4000	4100	4200	4300
Soybean meal	73,50	74,50	75,50	75,00	75,50
Wheat bran	10,00	8,00	6,00	7,60	8,50
Rice sauerkraut	8,00	5,00	4,00	4,00	2,50
Corn	1,00	2,00	4,00	3,00	2,00
Pequi oil	0,50	1,50	2,00	3,80	5,50
Dicalcium phosphate	1,65	1,65	1,65	1,65	1,65
Calcitic Limestone	0,97	0,97	0,97	0,97	0,97
Premix *	1,00	1,00	1,00	1,00	1,00
salt	0,40	0,40	0,40	0,40	0,40
Starch	0,50	3,75	4,50	2,54	2,00
B HT	0,04	0,04	0,04	0,04	0,04
Inert	2,44	1,18	0,00	0,00	0,00
Sum	100	100	100	100	100
Dry matter	86,94±0,09	86,77±0,35	91,66±0,11	91,52±0,20	91,86±0,17
Gross Prot%	41,87±0,26	41,07±0,03	38,82±1,21	41,12±0,28	41,45±0,41
Kcal / kg	3900	4000	4100	4200	4300
Brute Fiber	21,09±0,80	21,21±1,44	14,74±1,08	12,82±0,91	11,16±2,65
Ethereal Extract	2,15±0,60	2,57±0,21	3,70±0,43	5,38±0,78	8,46±1,88

*Commercial mineral e vitamin premix (5 kg ton^{-1}), with guaranteed levels to the gram scale: Vit. A, 1.200.000 UI; Vit. D3, 200.000 UI; Vit k3, 2.400 mg; Vit B3. 4.800 mg; Vit B2 , 4.800 mg, Vit B6, 4.000 mg, Vit B12, 4.800 mg,. Pholic acid, 1.200 mg; pantotenato Ca 12.000mg; Vit. C, 48.000 mg; biotine, 48 mg; coline choret , 108.000 mg; niacine, 24.000 mg; and commercial mineral premix (1 kg ton^{-1}), with guaranteed levels Fe, 50.000 mg; Cu, 3.000 mg; 20.000 mg; Mn, 20.000 mg; Zn, 3.000 mg; I, 100 mg; Co, 10 mg; Se, 100 mg.

The chemical-bromatological composition of the experimental diets was determined at the Food Laboratory of the Faculty of Agronomy and Veterinary Medicine of the University of Brasilia (UnB) and is shown in Table 1.

Environmental conditions and supply of diets

Temperature, dissolved oxygen, and pH of the water were monitored daily with a portable meter (Oximeter SL 520 microprocessor, Brazil). Ammonia was weekly measured (Nesller's method). The fish were fed twice a day (at 8:00 and 17:00), until apparent satiation. At the end of the experimental period (60 days), the fish were fasted for 12 hours to empty their digestive system. To perform the biometrics (initial and final), blood and digestive system collection, the animals were previously desensitized in a benzocaine solution (1:10,000).

Performance evaluation

To evaluate the performance, estimated consumption (EC), survival rate (TS), weight gain (GP), feed conversion (CA), specific growth rate (TCE), protein efficiency rate (TEP), index hepatosomatic index (IHS) and digestive-somatic index (DIS) were measured according to the following equations:

Formulas:

1. TS (%) = (Final number of individuals / Initial number of individuals) x 100
2. GP = Final weight – Initial weight
3. CA = Total Consumption / Weight Gain
4. TCE (% d-1) = [(Final weight – Starting weight) / time] x 100
5. TEP = (Weight Gain / Total Protein Intake) x 100
6. IHS (%) = (Liver Weight / Body Weight) x 100
7. IDS (%) = (Digestive System Weight / Body Weight) x 100

At the end of the experimental period, the animals were separated, collected, and euthanized. The samples were then stored in plastic bags and frozen (-20ºC) until analyzed.

The carcass samples were later sent to the Food Laboratory of the Faculty of Agronomy and Veterinary Medicine at UnB, where they were dried in a ventilated oven at 40ºC for 48 hours, and ground. The samples were then analyzed for their composition in dry matter, crude protein, and ether extract according to procedures described by Silva (1990).

Hematological parameters

At the end of the experimental period, four fish were randomly collected from each box for blood collection by puncture of the caudal vein with syringes previously bathed with anticoagulant (3% EDTA). Blood samples were separated into two aliquots in "Eppendorf" tubes, one with an anticoagulant to determine the hematological parameters and the other one without, intended to obtain serum for analysis of total proteins, albumin, and serum globulins. A small fraction of the blood was used to make blood smears stained with Panotic (Interlab) for differential counting of total leukocytes (Hrubec & Smith, 1998).

Blood samples were used to determine the hematocrit value by the microhematocrit technique (Goldenfarb, Bowyer, Hall, & Brosious, 1971), hemoglobin measurement by the cyanmethemoglobin method (Collier, 1944), and erythrocyte count in a Neubauer chamber using Hayen's liquid diluent. From the results obtained, the hematimetric indices were calculated, comprising the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCM), and Mean Corpuscular Hemoglobin Concentration (CHCM) according to the formulas:

8. MCV (fL) = (Hematocrit x 10) / number of erythrocytes
9. HCM (pg) = (Hemoglobin rate x 10) / number of erythrocytes
10. CHCM (g dL^{-1}) = (Hemoglobin rate x 100) / Hematocrit

The serum globulin concentration value was achieved through the indirect method by subtracting the value of serum total protein from albumin. For this, the serum will be separated by centrifugation, and both serum protein and serum albumin are determined by colorimetric assays in a spectrophotometer (Hach DR 6000, 2013, Germany) using commercial kits (Labtest, Brazil).

Statistical analysis

The results were submitted for analysis of variance (ANOVA) and polynomial regression. The parameters that presented significant differences had their means compared by the Duncan test, at a 5% probability. The data were analyzed using the SAS statistical program.

Results

During the experimental period, the mean temperature and dissolved oxygen values of the water were 26.80 ± 1.97ºC and 9.6 ± 1.24 mg L^{-1}, respectively. The pH was 6.3 ± 0.25, and ammonia 0.003 ± 0.001 mg L^{-1}.

The performance results are available in Table 2. After the 60-day trial period, good body condition and a 100% survival rate were observed. Significant differences ($p < 0,05$) were observed in the estimated consumption, and no significant differences were observed for the parameters gain of weight (GP) and feed conversion (CA). Specific growth rates presented statistical differences ($p < 0,05$). No significant differences were observed in protein efficiency rates ($p > 0,05$).

The hepatosomatic index (IHS) was higher in the animals with a higher level of added oil when compared to the other groups ($p < 0,05$), presenting linear behavior (Figure 1). For somatic digestive index (SDI), significant differences were observed ($p > 0,05$) between the lower level of added oil and other treatment. IDS presented a linear regression model for the inclusion of the oil (Figure 1).

Table 2. Performance, feed efficiency and biological indexes of tambaqui juveniles (*C. macropomum*) fed with different levels of pequi oil (*C. brasiliense*, Camb) for 60 days.

	E.B Kcal kg^{-1}					
Parameters[a]	3900	4000	4100	4200	4300	CV%
TS (%)	100	100	100	100	100	
CE (g)	13.08±4.57a	11.16±4.48c	11.84±4.58bc	12.57±4.69ab	11.68±4.68bc	38.05
GP (g)	50.05±14.94	37.79±15.17	39.10±13.10	44.26±11.57	38.03±15.05	34.85
CA	1.32±0.51	1.53±0.80	1.43±0.42	1.32±0.36	1.46±0.5	39.34
TCE	100.10±29.87a	75.59±30.33b	78.20±26.21b	88.51±23.14ab	76.05±30.09b	15.03
TEP	84.91±25.64	79.67±31.98	76.08±22.71	81.03±21.50	72.60±30.57	15.26
IHS	0.0154±0.0025b	0.0161±0.0041b	0.0137±0.0019b	0.0139±0.0017b	0.4686±0.0695a	31.92
IDS	0.0335±0.0043a	0.0348±0.0043b	0.0284±0.0024bc	0.0272±0.0032c	0.0280±0.0025bc	16.15

[a]Valores de média ± Desvio Padrão. Letras diferentes na mesma linha significam diferenças estatísticas entre si pelo teste Duncan ($p < 0.05$). CV - Coefficient of variation, TS - Survival rate, EC - Estimated consumption, GP - Weight gain, CA - Food Conversion, TCE - Specific growth rate, IHS - hepato-somatic index, IDS - Digestive-Somatic Index

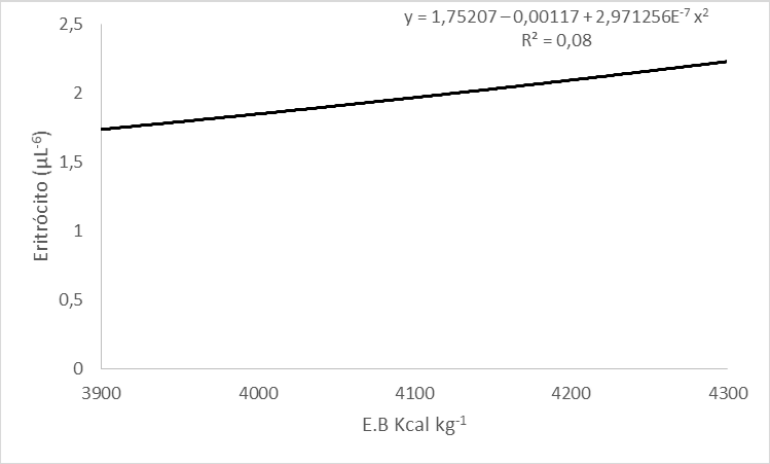

Figure 1. Regression equation for the levels of pequi oil addition (C. brasiliense) in tambaqui juveniles (C. macropomum) for 60 days for hepato-somatic index and digestive-somatic index

The determination of the chemical composition of the carcasses of the animals submitted to the treatments is shown in Table 3. No differences ($p > 0,05$) were observed in dry matter. Significant differences ($p < 0,05$) in crude protein and ethereal extract were observed for the inclusion of pequi oil levels. Adding increasing levels of the test lipid source resulted in increased fat deposition in the carcass of tambaqui (*Colossoma macropomum*) juveniles.

The results of hematological parameters and differential count of leukocytes are presented in Table 4. Significant differences were not observed for hematocrit values during the experimental period. Erythrocytes showed significant differences ($p < 0,05$) and quadratic regression (Figure 2). The highest value for erythrocyte quantity was obtained for the last level of adding the test oil. Hemoglobin value was statistically different ($p < 0,05$). Significant differences ($p < 0,05$) for the hematimetric indices (VCM, HCM, and CHCM) were also obtained in the study.

Table 3. Chemical composition of tambaqui juveniles (*Colossoma macropomum*) fed with different levels of pequi oil (*C. brasiliense*, Camb) for 60 days.

Parameter[a]	E.B Kcal kg^{-1}					
	3900	4000	4100	4200	4300	CV(%)
Dry matter (%)	94.52±0.42	93.82±0.47	93.51±1.99	93.13±5.44	95.70±3.97	3.24
Crude Protein (%)	68.20±4.98a	65.29±8.71ab	58.91±13.45bc	55.01±7.19c	63.17±4.84ab	13.39
Ethereal extract	13.57±1.78a	17.04±1.24b	17.84±0.56bc	17.62±0.96bc	18.70±1.52c	7.30

[a]Values of mean ± standard deviation. Different letters on the same line mean statistical differences between them by the Duncan test ($p < 0.05$). CV - Coefficient of variation.

Table 4. Hematological parameters and differential count of leukocytes from tambaqui juveniles (C. macropomum) fed with different levels of pequi oil (C. brasiliense) at 60 days.

	E.B Kcal kg^{-1}					
Parameters[a]	3900	4000	4100	4200	4300	CV%
Hematocrit (%)	19.5±4.19	19.10±3.95	17.4±4.74	18.95±5.22	17.1±4.91	24.37
Erythrocytes (μL^{-6})	1.82±0.69a	1.86±0.58a	1.78±0.52a	2.10±0.65ab	2.34±0.71b	31.69
Hemoglobin (dL)	14.03±4.63a	10.30±3.53b	10.54±2.29b	13.34±3.24a	12.58±4.34ab	30.42
VCM (fL)	128.70±68.37a	112.48±61.18a	103.66±66.96ab	102.75±50.44ab	77.82±45.87b	41.50
HCM (pg)	90.72±51.47a	62.64±44.65b	63.95±41.91b	71.77±50.87ab	60.55±43.70b	47.61
CHCM (gdL^{-1})	74.10±26.4a	55.80±22.8b	63.20±20.3ab	74.10±22.5a	78.7±23.5a	33.02
Monocytes (µL)	6.31±3.33a	5.78±1.9a	13.54±5.8b	6.31±45.2a	16.69±9.2b	60.14
Lymphocytes (µL)	95.44±16.36	93.13±13.06	88.26±9.89	95.56±9.29	93.50±10.17	11.66
CGE (µL)	5.76±3.69a	2.71±1.59b	2.03±1.33b	3.54±2.17ab	3.44±2.87ab	72.17
Neutrophils (µL)	18.25±15.90	20.07±64.00	21.38±9.03	19.44±5.99	12.63±8.64	57.94

[a]Values of mean ± standard deviation. Different letters on the same line mean statistical differences between them by the Duncan test ($p < 0.05$). CV - Coefficient of variation, VCM - Mean corpuscular volume, HCM - Mean corpuscular hemoglobin, CHCM - Mean corpuscular hemoglobin concentration, CGE - Special granulocytic cell.

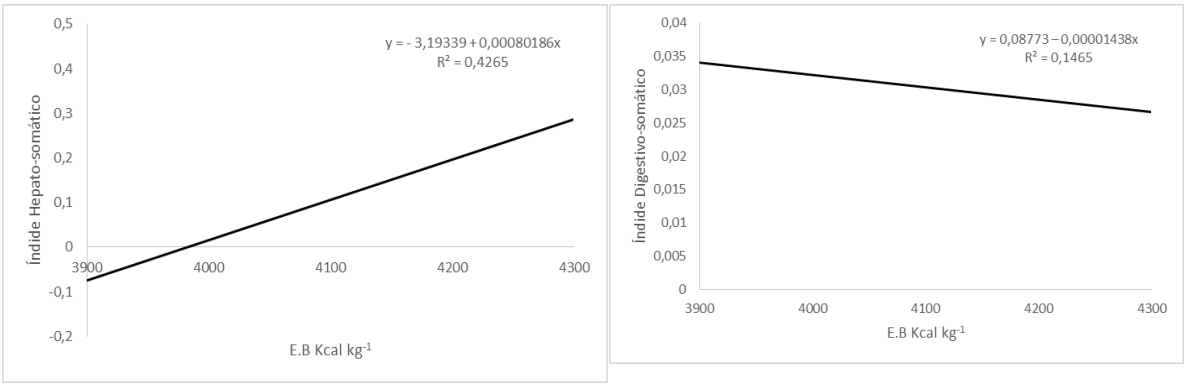

Figure 2. Regression equation for pequi oil addition levels (C. brasiliense) in tambaqui juveniles (C. macropomum) for 60 days for erythrocyte.

Monocyte volume was significantly different ($p > 0,05$), presenting a linear regression model (Figure 3). The highest amount observed occurred at the maximum level of pequi oil added, which did not differ statistically from the intermediate treatment. The granulocytic cells were significantly different ($p > 0,05$) between treatments. There was no difference in lymphocyte and neutrophil volumes between treatments, however, both presented a quadratic regression model (Figure 4).

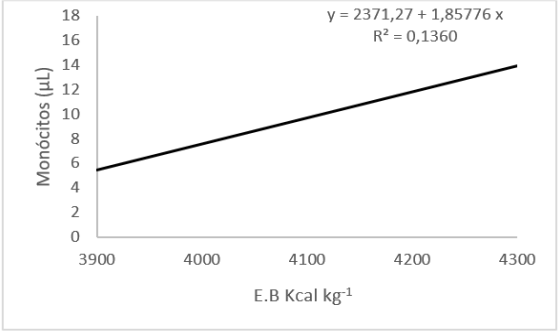

Figure 3. Regression equation for pequi oil addition levels (C. brasiliense, Camb) in tambaqui juveniles (C. macropomum) for 60 days for variable monocytes.

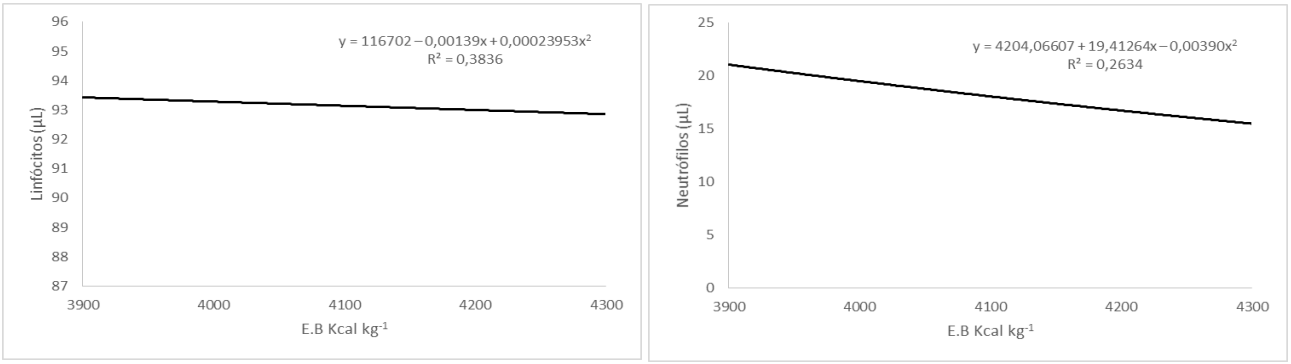

Figure 4. Regression equation for pequi oil addition levels (C. brasiliense, Camb) in tambaqui juveniles (C. macropomum) for 60 days for lymphocyte and neutrophil variables.

The serum protein showed a quadratic effect (Figure 5), displaying a statistical difference ($p > 0,05$) between the treatments. A lower volume of total serum protein was observed in the treatment that received less oil. Albumin and globulin were also significantly different ($p > 0,05$), and both parameters showed a quadratic effect (Figure 6) (Table 5). The leukocytes identified in the experiments were monocytes, lymphocytes, granulocytic cells, and neutrophils (Figure 7).

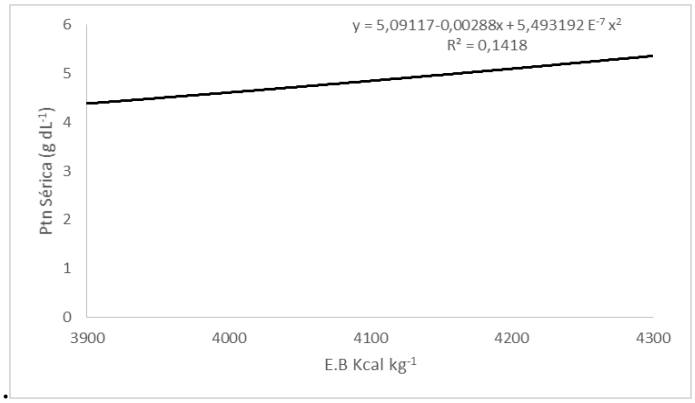

Figure 5. Regression equation for pequi oil addition levels (C. brasiliense, Camb) in tambaqui juveniles (C. macropomum) for 60 days for total serum protein variable.

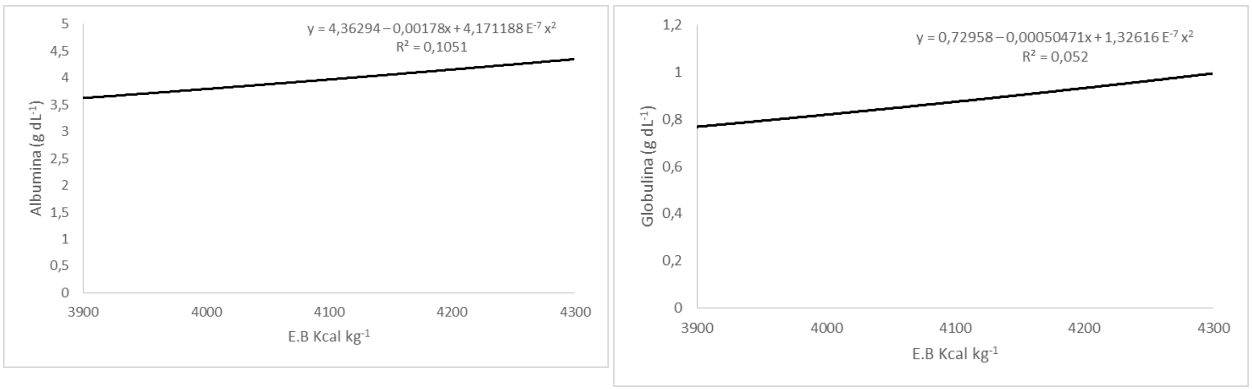

Figura 6. Equação de regressão para os níveis de adição de óleo de pequi (*C. brasiliense*, Camb) em juvenis de tambaqui (C. *macropomum*) por 60 dias para variáveis albunima e globulina.

Table 5. Total protein, albumin and globulin in tambaqui juveniles (C. macropomum) fed with different levels of pequi oil (C. brasiliense) a.

	E.B Kcal kg^{-1}					
Parameters[a]	3900	4000	4100	4200	4300	CV%
Serum Protein (g dL^{-1})	4.10±1.11b	4.95±0.517a	4.97±0.52a	5.13±1.50a	5.18±0.94a	13.89
Albumin (g dL^{-1})	3.37±0.98c	3.95±0.51bc	4.27±0.49ab	4.25±1.31a	4.08±0.77ab	16.10
Globulin (g dL^{-1})	0.74±0.40cb	1.02±0.33ab	0.70±0.5a	0.88±0.33abc	1.10±0.40a	45.64

[a]Values of mean ± standard deviation. Different letters on the same line mean statistical differences between them by the Duncan test ($p < 0.05$). CV - Coefficient of variation.

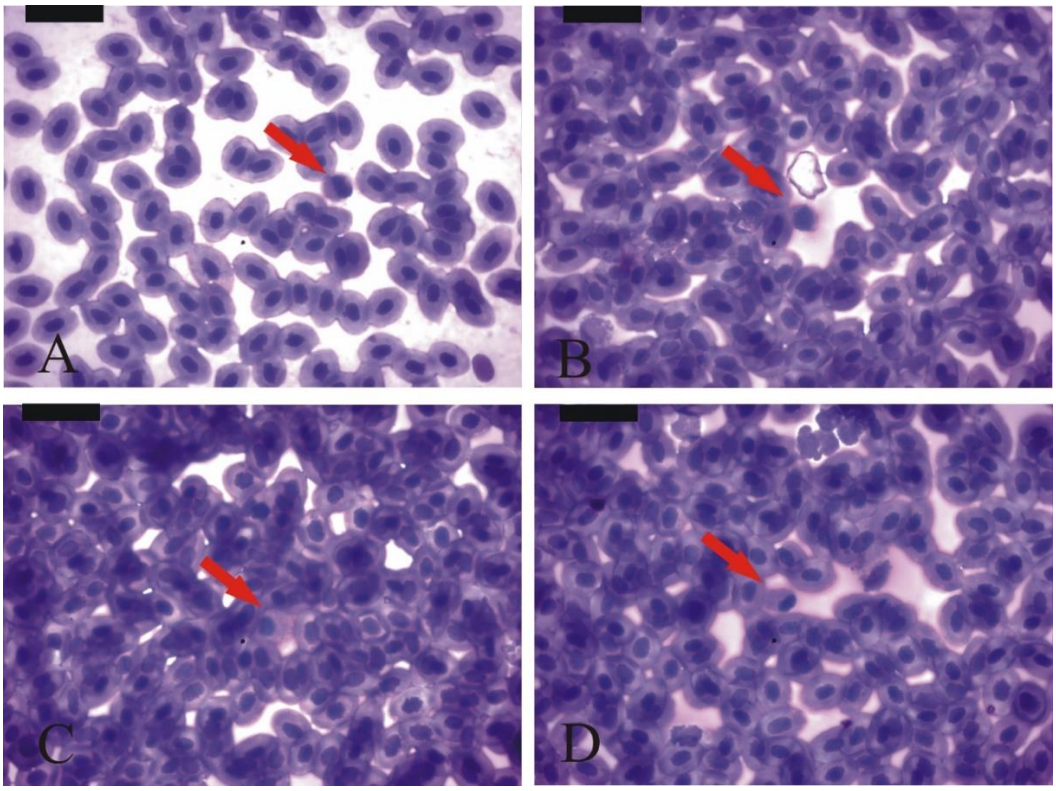

Figure 7. Leukocytes identified in tambaqui juveniles (C. macropomum) fed with pequi oil (C. brasiliense, Camb) for 60 days. (A) Monocyte; (B) Lymphocyte; (C) Special granulocytic cell; (D) Neutrophil. Coloration: Panótico; bar (4µm); increase (1000x).

Discussion

The water quality during the experimental period was within the recommended range for fish farming (Baldisseroto, 2002; Arana, 2004). The study showed no influence of the inclusion level of pequi pulp oil (C. brasiliense, CAMB.) on weight gain and feed conversion. No reports were found in the literature about the inclusion of pequi pulp oil in the diet of tambaqui (Colossoma macropomum) or other species.

The addition of pequi oil in increasing levels worsened the specific growth rate. The partial inclusion of linseed oil, rather than fish oil, did not compromise the performance of tilapia (Oreochromis niloticus) (Li, Lin, Lin, Chen, & Guan, 2016), Murray cod (Maccullochella peelii peelii.) (Francis, Turchini, Jones, & De Silva, 2006), and sharpsnout seabream (Diplodus puntazoo) (Piedecausa, Mazón, García, & Hernández, 2007). The inclusion of corn and linseed oil did not affect the performance of juvenile silver catfish (Rhamdia quelen) (Vargas et al., 2008).

For the variable protein efficiency rate (TEP) no influence of treatments was observed. Excess n-6 series can cause inhibition of n-3 PUFA conversion (Horrobin, 1991; Shils, Shike, Ross, Caballero, & Cousins, 2003), resulting in altered lipid metabolism, fat deposition, and protein synthesis in muscle (Tirapegui, 2005), in addition to promoting changes in the composition of lipids in the carcass (Bell et al., 2001; Grisdale-Helland et al., 2002).

In the present study, a significant difference ($p < 0.05$) was observed for HSI with a higher value for the diet with 5.5 5 addition of oil and quadratic regression (Figure 1) as an effect of the levels of inclusion of pequi oil. A similar effect was observed in rainbow trout (Oncorhynchus mykiss) by the total inclusion of cottonseed oil and by diets consisting of a mixture of soybean oil and sunflower oil (Sener & Yildiz, 2003; Guler & Yildiz, 2011). According to Sargent et al. (2002) increased liver index is one of the symptoms of essential fatty acid deficiency and is indicative of n-6/n-3 imbalance (Robaina et al., 1998).

Due to the presence of n-3 and n-6 series PUFA in the natural diets of tambaqui, it is assumed that their supplementation is relevant to increased resistance to stress and diseases, and growth (Guimarães & Martins, 2015). The use of 5 – 20 g kg^{-1} of LNA (18:3 n-3) is recommended, based on total dietary lipid for tambaqui fish, with n-6 PUFA fatty acids being the most important (NRC, 2011).

The evaluation of hematological parameters is important and can indicate the health status of fish (Coles, 1986), being a good indicator of the degree of stress, disease, and diet influence (Mourente et al., 2005; Kader, Koshio, Ishikawa, Yokoyama, & Bulbull, 2010).

The inclusion of pequi pulp oil in the diet did not change the hematocrit in the present study. An increase in hematocrit was observed by Costa, Ferreira, Navarro, Rosa, and Murgas (2014) in Nile tilapia (Oreochromis niloticus) for diets containing soybean oil, and by Demir et al. (2014) for diets containing palm oil, also for Nile tilapia. However, Li, Lim, Klesius, and Welker (2013), in tilapia hybrids (O. niloticus x O. aureus), did not show significant differences in hematocrit for diets containing different levels of n-3 and n-6 PUFA.

The erythrocyte volume was higher in the treatment with the highest level of added vegetable oil and presented quadratic regression (Figure 2; Table 5). Tavares-Dias et al. (2009) indicates as reference values for hematocrit in C. macropomum, a minimum of 26% and a maximum of 38%, and for erythrocytes 1,250 and 2.96 µL-6, minimum and maximum, respectively. Despite the results of the present study for hematocrits being below the values indicated by Tavares-Dias et al. (2009), the erythrocyte values were within the reference values proposed by the aforementioned author.

The results obtained for hemoglobin (Table 5) by including the test lipid source remained within the reference values proposed by Tavares-Dias et al. (2009) (6.3-13.7 g dL^{-1}). The deficiency of essential fatty acids in juvenile Sparus aurata led to an increase in hemoglobin concentration as a consequence of reduced erythrocyte volume (Montero, Socorro, Tort, Caballero, & Robaina, 2004).

Erythrocytes and hematocrit are representatives of oxygen transport according to the number of circulating cells (Han et al., 2012), and the blood indices (MCV, MCH, and MCHC) are important in the diagnosis of anemia in most animals (Coles, 1986). According to Mccarthy, Stevenson, and Roberts (1973), the mean corpuscular hemoglobin concentration (MCHC) is the most accurate index in relation to the others, as it is calculated from the percentage of hematocrit and amount of hemoglobin, whereas the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCM) use the erythrocyte count, being more susceptible to error.

When comparing the results of CHCM from the present study (Table 5) with the reference (minimum of 20.2 and maximum of 30.5 g dL^{-1}) proposed by Tavares-Dias et al. (2009), it can be inferred that the inclusion of pequi pulp oil in the diet of juvenile tambaqui (C. macropomum) did not cause signs of anemia.

Tavares-Dias, Sandrim, & Campos-Filho, (1999) observed variation in the amounts of neutrophils (720 to 4240 µL) and lymphocytes (350 to 2489 µL) in juveniles of C. macropomum reared in an intensive system. The values obtained for neutrophils and lymphocytes in the present study are below the value found by Tavares-Dias et al. (1999). However, it cannot be suggested that this difference is caused by the treatments. Total leukocytes in teleosts show intraspecific variations influenced by the characteristics of each individual, in response to environmental stimuli (Negrete, Correa, Guevara, Atencio García, & Carrasco, 2009).

The values for monocytes and granulocytic cells in the study are within the range obtained by Tavares-Dias et al. (1999). White cells such as neutrophils and monocytes provide protection against pathogens (Harikrishnan et al., 2003). In the present study, no influence of the addition of pequi oil was observed in increasing levels for these two cell groups, so it can be inferred that there was no impairment of defense cells in juvenile tambaqui (C. macropomum).

According to Balfry et al. (2006), the fatty acids present in the diet of the fish act on the defense response in part due to the lipid composition of the membrane and its physical properties, since some defense responses are based on the interaction of leukocyte membranes, by activating the production of cytokines and also by the influencing the production of prostaglandin and leukotrienes by macrophages.

Prostaglandins, especially PGE2, are derived from arachidonic acid (ARA). These are produced by monocytes and are associated with modulating immune cell function (Bell & Sargent 2003, Yaqoob, 2004). According to Ganga et al. (2005), diets rich in n-6 PUFA result in higher amounts of PGE2 than diets rich in n-3 PUFA. It can be inferred that the increase in monocytes in the present study is related to the presence of linoleic acid (LA) in pequi oil.

Total protein concentration is also a function of nutritional status (Igwebuike, Anugwa, Raji, Ehiobu, & Ikurior, 2008) and is a usual indicator of the immune system in fish (Demir et al., 2014), where its increase is associated with a strong innate response in fish. (Andrews, Sahu, Pal, Mukherjee, & Kumar, 2011). In the present study, the addition of pequi pulp oil resulted in positive quadratic regression (Figure 5) for total serum protein for juvenile tambaqui (C. macropomum). A similar result was observed by Demir et al. (2014) in Nile tilapia (Oreochromis niloticus) in diets with palm oil. The inclusion of peppermint oil (M. piperita) in juveniles of C. macropomum kept total protein levels constant (Ribeiro et al., 2016).

The main plasma proteins are albumin and globulin (Melo, Oliveira, Júnior, Teixeira, & Guimarães, 2009), and albumin synthesis is influenced by nutrition, hormonal balance, general liver status, and stress (Hasegawa,

Fonteque, Kohayagawa, & Boretti**,** 2002). Increased levels of total proteins and serum globulin are an indication that the fish is immunologically resistant (Nayak, Das, Kohli, & Mukherjee, 2004). Thus, it can be predicted that pequi pulp oil has the potential to act as an immunonutrient for tambaqui juveniles (C. macropomum).

The fish submitted to the treatments showed increasing levels, in a positive quadratic way (Figure 6), for albumin globulin when fed with increasing levels of pequi pulp oil (Caryocar brasiliense). Elevated albumin levels may be related to osmoregulatory dysfunction or damage to surrounding blood vessels (Demir et al., 2014). However, no hematological changes were observed to confirm that the increase in albumin caused harm to the animals.

Conclusion

The addition of pequi oil (Caryocar brasiliense, Camb.) in the diet of tambaqui (*Colossoma macropomum*) juveniles reduced the specific growth rate and increased hepatic fat deposition. However, no impairment on the health of the animals was observed. It is not recommended to use pequi oil, within the limits tested, as a lipid source for tambaqui juveniles in order to ensure good zootechnical performance.

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