

GROWTH PERFORMANCE AND CHEMICAL COMPOSITION OF PACUS (*Piaractus mesopotamicus*) FED WITH VEGETABLE OIL SOURCES AND ALPHA-LIPOIC ACID*

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ABSTRACT

The objective of this study was to evaluate the effects of different oil sources and alpha-lipoic acid levels on pacu performance, chemical composition of fillets and histological changes in the liver. Were used 480 pacu juveniles (3.35 ± 0.78 g), distributed in 24 experimental units ($n = 20$). Six treatments were delineated in a completely randomized design (CRD), in a 3x2 factorial scheme, with three sources of oil (soybean, sesame and linseed) and two levels of alpha-lipoic acid (0 and 0.1 %), with four replications. Data were analyzed statistically by analysis of variance (ANOVA) and Tukey's test (5%). Diets containing linseed oil as the only lipid source provided better indices of final body weight and individual weight gain in pacu juveniles. Crude protein content was higher in fish fillets that fed diets without alpha-lipoic acid. Liver's histology was not affected by the experimental diets. In conclusion, linseed oil improved final body weight and individual weight gain on pacus, and alpha-lipoic acid did not affect the performance of the animals, however its absence promoted higher levels of crude protein in fish fillets.

Keywords: average weight; crude protein; individual weight gain; linseed; liver; sesame

DESEMPENHO PRODUTIVO E COMPOSIÇÃO QUÍMICA DE PACUS (*Piaractus mesopotamicus*) ALIMENTADOS COM ÓLEOS VEGETAIS E ÁCIDO ALFA-LIPOICO

RESUMO

O objetivo do estudo foi avaliar os efeitos de óleos vegetais e níveis de ácido alfa-lipoico sobre o desempenho de pacus, composição química de filés e alterações histológicas no fígado. Foram utilizados 480 juvenis de pacu ($3,35 \pm 0,78$ g), distribuídos em 24 unidades experimentais ($n = 20$). Foram testados seis tratamentos em delineamento inteiramente casualizado (DIC), no esquema fatorial 3x2, sendo três fontes de óleo (soja, gergelim e linhaça) e dois níveis de ácido alfa-lipoico (0 e 0,1%), com quatro repetições. Os dados obtidos foram analisados estatisticamente por análise de variância (ANOVA) e teste de Tukey (5%). As dietas contendo óleo de linhaça como única fonte lipídica proporcionaram melhores índices de peso médio e ganho de peso individual nos juvenis de pacu. O teor de proteína bruta foi maior nos filés dos peixes alimentados com as dietas sem ácido alfa-lipoico. Os fígados de juvenis de pacu não foram afetados pelas dietas experimentais. Conclui-se que o óleo de linhaça promoveu os melhores índices de peso médio e ganho de peso individual em pacus e o ácido alfa-lipoico não afetou o desempenho dos animais, mas a sua ausência promoveu maiores teores de proteína bruta nos filés dos pacus.

Palavras-chave: peso médio; proteína bruta; fígado; ganho de peso individual; linhaça; fígado; gergelim

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INTRODUCTION

The addition of lipids, from vegetable or animal sources, in fish diets is common. In general, vegetable oils, especially soybean oils, are used, and the quality of these products is assessed primarily in relation to nutritional quality (DUNFORD, 2004). Besides playing an important role in food quality, lipids are extremely important for living organisms in general (PORTZ and FURUYA, 2013), as they participate in the formation and maintenance of various tissues, organs and structures, as well as being sources of essential fatty acids (SARGENT *et al.*, 2003). In addition, the amount and/or quality of the lipids in the diets may influence the structure of the hepatocytes of the fish livers (CABALLERO *et al.*, 1999) and even the hepatosomatic index of the animals (CHEN *et al.*, 2013).

For fish, lipids are the main source of energy, releasing more available energy than proteins and carbohydrates (GLENCROSS, 2009). There are several factors that may influence the optimum level of lipids needed in fish diets, such as the species and environmental conditions to which they are adapted (fresh water or salt water, water temperature), food habits (carnivorous or omnivore/herbivore), development phase (larvae, juveniles, adults or breeding), and the amount of proteins and carbohydrates present in the diet due to the interactions between lipid, carbohydrate and protein metabolism (GARCIA *et al.*, 2013), and the lipid source to be used (ZHOU *et al.*, 2014).

The vegetable oils that are used in fish feed are soybean oil and linseed oil. Soybean oil, which is rich in linoleic acid (MCCLEMENTS and DECKER, 2010), can be used as a source of n-6 fatty acid, and linseed oil, which is rich in linolenic acid (MCCLEMENTS and DECKER, 2010), can be used as source of n-3 fatty acid (REGOST *et al.*, 2003). Sesame oil is known to exhibit high oxidative stability due to the antioxidant properties of bioactive compounds present in its composition, such as sesamin and sesamol (KOCHHAR, 2002). High concentrations (5.8 g kg⁻¹) of sesamin in salmon diets are capable of affecting the energy metabolism of fish, especially the metabolism of liver and white muscle (WAGNER *et al.*, 2014).

Alpha-lipoic acid (ALA) is also a bioactive compound, found in green leaves (spinach, broccoli), potato, barley, wheat germ, red meat, rice and derivatives, among others (PORTELA *et al.*, 2014), and presents neuroprotective and anti-inflammatory properties (HOLMQUIST *et al.*, 2007).

Among native species, pacu (*Piaractus mesopotamicus*) has stood out in recent years as a species of great economic interest in Brazil. As well as its excellent characteristic of adapting to the climatic conditions, it has omnivorous food habit, with strong tendency to herbivorous, as it is able to feed on fruits, crustaceans, organic debris, small fishes and molluscs (URBINATI *et al.*, 2005).

In pacu juveniles fed with diets supplemented with ALA (1,000 mg kg⁻¹ of feed) and/or ascorbate (500 mg AA kg⁻¹ of feed), eicosapentaenoic acid (EPA, 20:5n-3) levels increased significantly in the muscles, but not significantly in the brain, suggesting that the brains are influenced very little by supplementation of this substance in the diet, a fact that can be explained by a possible defensive reaction of these animals to environmental changes (TRATTNER *et al.*, 2007).

In view of the importance of the use of vegetable oil sources and bioactive components, such as alpha-lipoic acid, in fish nutrition, this study aimed to evaluate the effects of adding of the three sources of vegetable oils (soybean, sesame and linseed) and the two concentrations of alpha-lipoic acid on the performance and chemical composition of fillets and histological changes of the liver of pacus.

MATERIAL AND METHODS

The experiment was approved by the Research Ethics Committee of the Faculty of Animal Science and Food Engineering (N° 5451281015). The experimental period lasted for 90 days, using 480 pacu juveniles (3.35 ± 0.78 g) from a commercial fish farm.

Local

The trial was conducted at the Paulista Agribusiness Technology Agency (APTA, Pirassununga-SP). The analyses of the chemical composition of the diets and muscles and the

histology of the livers were carried out at the Faculty of Animal Science and Food Engineering, University of São Paulo (FZEA/USP).

Experimental design

Six treatments were distributed in a completely randomized design (CRD), in a 3x2 factorial scheme, with three sources of vegetable oils (soybean, sesame and linseed) and two levels of alpha-lipoic acid (0 and 0.1%), with four replicates. Each treatment corresponded to an experimental diet and was calculated in relation to the total percentage of the diet, as described below: T1 (control diet) - 5% soybean oil (SBO); T2 - 2.5% sesame oil (SO) + 2.5% linseed oil (LO); T3 - 5% LO; T4 - 5% SBO + 0.1% alpha-lipoic acid (ALA); T5 - 2.5% SO + 2.5% LO + 0.1% ALA; T6 - 5% LO + 0.1% ALA.

Experimental diets

The analysis of the chemical composition of the ingredients and experimental diets were performed in triplicate, according to the AOAC (2000) methodology: dry matter was obtained after drying the samples in an oven at 105 °C until constant weight; crude protein was obtained by the micro-Kjeldahl method, after acid digestion (total nitrogen x 6.25); ether extract was determined by extraction by heating the petroleum ether in a Soxhlet apparatus for six hours; mineral content was obtained by incineration in a muffle furnace at 550 °C for four hours; and gross energy was determined in a calorimetric pump.

After previous analysis of the chemical composition of the ingredients, six isoproteic (22% crude protein - CP) and isoenergetic (4,200 kcal.kg⁻¹ of GE; gross energy) diets were formulated as presented in Table 1.

In order to prepare the experimental diets, the ingredients were ground, weighed, homogenized and submitted to the extrusion process (2 mm granulometry) using an extruder with a processing capacity of 30 kg hour⁻¹ (NX 30 Imbramaq, Ribeirão Preto-SP, Brazil) at Fisheries Laboratory of ESALQ - USP (Piracicaba-SP). After extrusion, soybean, linseed and sesame oils, as well as alpha-lipoic acid, were added in the proportions of each treatment and homogenated. After drying in a forced ventilation oven (55 °C

for 24 h), the diets were conditioned in identified plastic containers and stored in a cold room (4 °C) until use.

Fish and experimental conditions

Prior to the beginning of the experiment, fish were acclimatized to experimental conditions for seven days and fed with a commercial diet (Pirá Alevinos 1.7 mm, Guabi Nutrição Animal, Campinas-SP, Brazil) with 40% crude protein, which had been ground in a meat grinder, and the granules retained in a 1 millimeter mesh were selected. After the acclimatization period, fish were individually weighed (3.35 ± 0.78 g) and randomly distributed in 24 tanks with a capacity of 50 L, in a recirculating system with thermostatic heaters and a biological filter. Each tank corresponded to an experimental unit.

The parameters of water quality evaluated were temperature (T), dissolved oxygen (DO), hydrogenation potential (pH), nitrite (NO₂⁻) and toxic ammonia (NH₃). Temperature was measured with a digital thermometer (Incoterm, Porto Alegre-RS, Brazil) with minimum and maximum daily temperature records. The other water parameters were evaluated weekly throughout the experimental period. Dissolved oxygen was measured with a digital oximeter (YSI Pro2, Yellow Springs Instruments, Ohio, USA), and pH, nitrite and toxic ammonia were measured daily using commercial kits (Labcon Test). The animals were fed *ad libitum* until apparent satiation twice a day (8:00 a.m. and 5:00 p.m.) for 90 days.

Two individual biometrics were performed, one before starting the administration of the experimental diets (Day 0) and another at the end of the experimental period (Day 90), in order to measure the parameters of growth performance of the pacus. In the first biometry, the fish were anesthetized with eugenol (20 mg L⁻¹). In the second biometry, the fish were immersed in a lethal dose (250 mg L⁻¹) of benzocaine (BRASIL, 2013). Six fish per experimental unit were filleted and the fillets were frozen separately in polyethylene packages and stored in a freezer (-20 °C) for further analysis.

Growth performance

To evaluate the efficiency of the experimental diets, some growth performance parameters were

determined: final body weight (FBW), individual weight gain (IWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and survival rate (SR) - according to the formulas described below (NRC, 2013): FBW = total biomass/number of animals;

IWG = final weight - initial weight; FI = total feed intake per experimental unit; FCR = FI/IWG; PER = [FBW/(FI x crude protein of diet)] x 100; SGR = [(log final weight - log initial weight)/time (days)] x 100; SR = [(number of alive animals - number of dead animals)/number of total animals] x 100.

Table 1. Ingredients (%) and chemical compositions (dry basis; %) of experimental diets for pacu juveniles. T1 (control diet) - 5% soybean oil (SBO); T2 - 2.5% sesame oil (SO) + 2.5% linseed oil (LO); T3 - 5% LO; T4 - 5% SBO + 0.1% alpha-lipoic acid (ALA); T5 - 2.5% SO + 2.5% LO + 0.1% ALA; T6 - 5% LO + 0.1% ALA.

Ingredient (%)	T1	T2	T3	T4	T5	T6
Soybean oil	5.00	0.00	0.00	5.00	0.00	0.00
Sesame oil	0.00	2.50	0.00	0.00	2.50	0.00
Linseed oil	0.00	2.50	5.00	0.00	2.50	5.00
Alpha-lipoic acid	0.00	0.00	0.00	0.10	0.10	0.10
Corn gluten meal	0.18	0.18	0.18	0.18	0.18	0.18
Soybean meal	24.65	24.65	24.65	24.65	24.65	24.65
Wheat meal	25.30	25.30	25.30	25.30	25.30	25.30
Broken rice	11.44	11.44	11.44	11.44	11.44	11.44
Ground corn	25.75	25.75	25.75	25.75	25.75	25.75
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Premixed vitamins and minerals ¹	1.00	1.00	1.00	1.00	1.00	1.00
BHT	0.05	0.05	0.05	0.05	0.05	0.05
Corn starch	4.21	4.21	4.21	4.21	4.21	4.21
Lysine	0.82	0.82	0.82	0.82	0.82	0.82
Sand (inert)	0.10	0.10	0.10	0.00	0.00	0.00
Chemical composition						
DM (%)	90.38	90.81	90.43	90.86	90.42	91.14
CP (%)	22.23	22.28	22.15	22.21	22.35	22.20
EE (%)	5.07	5.02	5.02	5.06	5.05	5.02
MM (%)	4.29	4.28	4.32	4.31	4.29	4.30
GE (kcal kg ⁻¹)	4,202	4,201	4,205	4,204	4,202	4,202

¹ Composition of premixed vitamins and minerals: vit. A - 500,000 UI; vit. D3 - 250,000 UI; vit. E - 5,000 mg; vit. K3 - 500 mg; vit. B1 - 1,500 mg; vit. B2 - 1,500 mg; vit. B6 - 1,500 mg; vit. B12 - 4,000 mg; folic acid - 500 mg; pantothenate Ca - 4,000 mg; vit. C - 10,000 mg; biotin - 10 mg; Inositol - 1,000; nicotinamide - 7,000; choline - 10,000 mg; Co - 10 mg; Cu - 1,000 mg; Fe - 5,000 mg; I - 200 mg; Mn - 1,500 mg; Se - 30 mg; Zn - 9,000 mg³. BHT: Butylated hydroxytoluene; DM: dry matter; CP: crude protein; EE: ether extract; MM: mineral matter; GE: gross energy.

Chemical composition of pacu fillets

For each experimental unit, three fish were randomly collected, filleted, stored in polyethylene packages in a freezer (-20 °C) and kept frozen until the analysis. Analyses of the chemical composition of fish fillets were carried out in triplicate according to the AOAC (2000) methodology: moisture, crude protein, ether extract and mineral matter, as previously described.

Liver histology

At the end of the experimental period, livers from three fish per experimental unit were collected and stored frozen at -20 °C for further analysis. After thawing, the samples were fixed in Bouin for 24 hours and then stored in 70% alcohol. The blocks were made with paraffin and histological sections were made using a microtome, with a thickness of 5 µm. In order to evaluate the morphology of the structures under light

microscopy and to measure the area of the epithelial mucosa, histological sections were dewaxed, rehydrated according to routine histological methods and submitted to staining with hematoxylin and eosin (BANCROFT and GAMBLE, 2008). The slides were photographed using a light microscope (Carl Zeiss, Germany) coupled to a digital camera and analyzed with the aid of Axio Vision 4.1 software.

Statistical analysis

The data were analyzed using Statistical Analysis System (SAS, 2011), version 9.3, by analysis of variance ANOVA and significant differences were verified by Tukey's Test with a significance of 5%. Means within each factor (oil sources and ALA levels) were compared by ANOVA and Tukey's Test (5%). In the analyses of variance, SAS procedure PROC GLM was used.

All assumptions of the statistical model were verified using SAS/LAB of SAS (R). When the assumption of homogeneity of variance was rejected, SAS procedure PROC GLIMMIX was used by associating different variances with each treatment. When the assumptions were satisfied, SAS procedure PROC GLM was used.

RESULTS

Growth performance

Growth performance data are presented in Table 2, and there were no differences ($P>0.05$) among the treatments evaluated.

Although no differences were observed between the treatments for growth performance ($P>0.05$), there was a difference between FBW and IWG within each factor (oil sources and ALA levels) (Table 3).

Table 2. Parameters of growth performance (mean \pm standard deviation) of pacu juveniles fed different experimental diets after 90 days. T1 (control diet) - 5% soybean oil (SBO); T2 - 2.5% sesame oil (SO) + 2.5% linseed oil (LO); T3 - 5% LO; T4 - 5% SBO + 0.1% alpha-lipoic acid (ALA); T5 - 2.5% SO + 2.5% LO + 0.1% ALA; T6 - 5% LO + 0.1% ALA.

Variable	T1	T2	T3	T4	T5	T6
FBW (g)	50.12 \pm 7.09	52.63 \pm 3.80	56.84 \pm 7.62	48.08 \pm 6.60	53.15 \pm 6.02	60.65 \pm 8.91
IWG (g)	46.85 \pm 7.04	49.11 \pm 3.67	53.49 \pm 7.75	44.72 \pm 6.51	49.89 \pm 5.92	57.33 \pm 8.92
FI (g)	66.84 \pm 9.91	71.06 \pm 8.08	74.62 \pm 8.98	65.78 \pm 9.06	68.92 \pm 7.55	75.22 \pm 9.20
FCR	1.43 \pm 0.10	1.45 \pm 0.11	1.40 \pm 0.08	1.47 \pm 0.07	1.38 \pm 0.08	1.32 \pm 0.05
PER (%)	3.16 \pm 0.22	3.12 \pm 0.23	3.23 \pm 0.19	3.06 \pm 0.14	3.24 \pm 0.18	3.42 \pm 0.13
SGR (%.day ⁻¹)	1.32 \pm 0.07	1.31 \pm 0.03	1.37 \pm 0.07	1.36 \pm 0.13	1.35 \pm 0.04	1.40 \pm 0.07
SR (%)	88.75 \pm 13.15	87.50 \pm 11.90	90.00 \pm 10.80	88.75 \pm 6.29	97.50 \pm 2.89	86.25 \pm 15.48

FBW: final body weight; IWG: individual weight gain; FI: feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; SGR: specific growth rate; SR: survival rate.

Table 3. Mean values of final body weight (FBW) and individual weight gain (IWG) of pacu juveniles fed different experimental diets, within each factor (oil sources and ALA levels).

Variable	Oil Sources			P-value	Levels of ALA		P-value
	SBO	SO+LO	LO		0%	0.1%	
FBW (g)	49.10 ^b	52.89 ^{ab}	58.74 ^a	0.04*	53.20	53.96	0.79 ^{ns}
IWG (g)	45.78 ^b	49.50 ^{ab}	55.41 ^a	0.04*	49.81	50.65	0.77 ^{ns}

FBW: final body weight; IWG: individual weight gain; SBO: soybean oil; SO+LO: sesame oil + linseed oil; LO: linseed oil; ALA: alpha-lipoic acid; ^{ns} non-significant ($P>0.05$); * significant ($P<0.05$). Means of the same factor followed by different letters in the same row differ from each other ($P<0.05$).

Chemical composition of pacu fillets

Analyses regarding the chemical composition of fish fillets can be seen in Table 4.

Although the chemical composition of the fillets did not present differences between the treatments ($P>0.05$), there was a significant difference between the means of CP within the factor of ALA levels.

The type of oil used in the diets did not influence ($P>0.05$) the CP levels of the fillets (16.34% CP mean). However, the use of ALA in the diet resulted in fillets with higher levels of CP (16.52%) than the 16.16% of CP of fillets of fish fed diets without ALA inclusion.

Liver histology

Evaluating the histology of the livers of fishes that received commercial diet, it was observed

that the hepatocytes had a polyhedral shape and normal arrangement of the nucleus, nucleolus and cytoplasm, with a rounded and central nucleus (which could be displaced to the periphery of the cell in some cases), well-evident nucleolus and clear cytoplasm (Figure 1), in accordance with what was observed by SOUZA *et al.* (2001) and FUJIMOTO *et al.* (2008) in their control groups. Also, it was possible to see the portal vein, which is responsible for draining blood into the liver, the cord-like arrangement of hepatocytes and sinusoids (coated by endothelial cells), which are irregularly distributed between hepatocytes (Figure 1).

Photomicrographs for the histological analysis of the livers of fish that received the experimental diets without alpha-lipoic acid (T1, T2 and T3) and supplemented with ALA (T4, T5 and T6) can be seen in Figures 2 and 3, respectively.

Table 4. Chemical composition (wet basis) (mean \pm standard deviation) of fillets of pacu juveniles fed different experimental diets. T1 (control diet) - 5% soybean oil (SBO); T2 - 2.5% sesame oil (SO) + 2.5% linseed oil (LO); T3 - 5% LO; T4 - 5% SBO + 0.1% alpha-lipoic acid (ALA); T5 - 2.5% SO + 2.5% LO + 0.1% ALA; T6 - 5% LO + 0.1% ALA.

Variable	T1	T2	T3	T4	T5	T6
H (g 100 g ⁻¹)	77.43 \pm 0.95	77.29 \pm 0.44	77.48 \pm 0.73	77.93 \pm 0.89	77.39 \pm 0.59	77.73 \pm 1.23
CP (g 100 g ⁻¹)	16.31 \pm 0.31	16.58 \pm 0.15	16.68 \pm 0.46	16.36 \pm 0.38	16.06 \pm 0.25	16.07 \pm 0.17
EE (g.100 g ⁻¹)	5.16 \pm 0.53	5.10 \pm 0.28	4.81 \pm 0.63	4.70 \pm 0.67	5.52 \pm 0.40	5.25 \pm 0.71
MM (g 100 g ⁻¹)	1.11 \pm 0.11	1.04 \pm 0.06	1.05 \pm 0.10	1.03 \pm 0.05	1.07 \pm 0.04	1.03 \pm 0.05

H: humidity; CP: crude protein; EE: ether extract; MM: mineral matter.

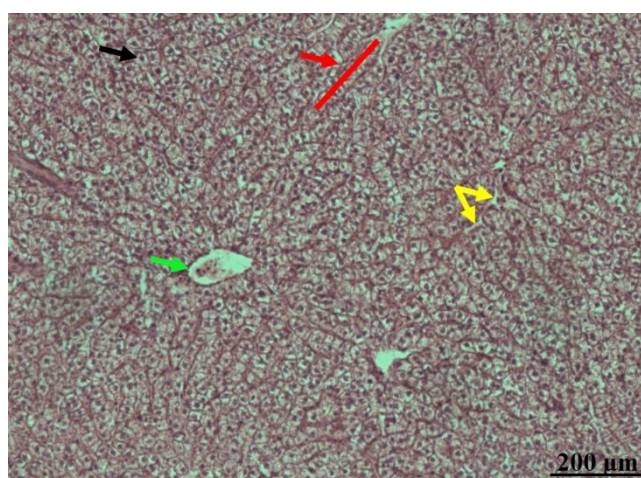


Figure 1. Histological photomicrograph (200 μ m) of liver of pacu fed with commercial diet, showing hepatocytes (black arrow), cordonal arrangement of hepatocytes (red arrow), sinusoids (yellow arrow) and portal vein (green arrow).

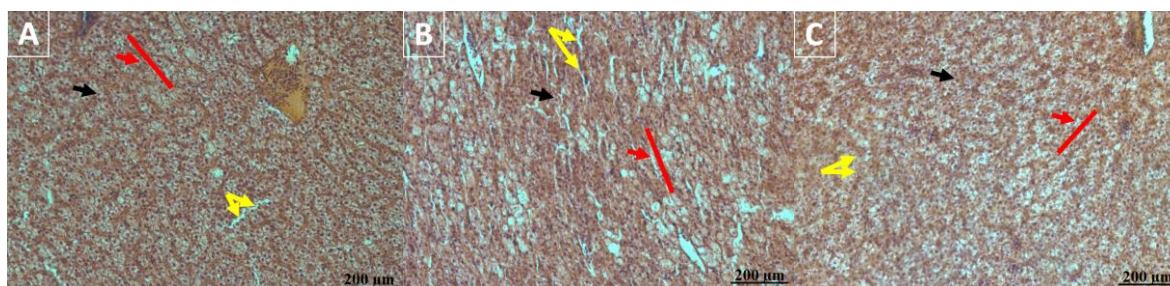


Figure 2. Histological photomicrographs (200 µm) of liver of pacu fed with the diets without alpha-lipoic acid: A) T1: soybean oil; B) T2: sesame oil + linseed oil; C) T3: linseed oil, evidencing hepatocytes (black arrow), cordonal arrangement of hepatocytes (red arrow) and sinusoids (yellow arrow).

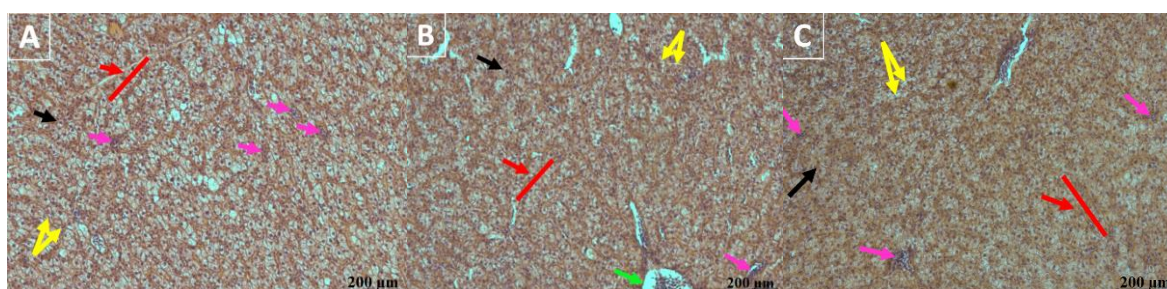


Figure 3. Histological photomicrographs (200 µm) of liver of pacu fed with diets supplemented with alpha-lipoic acid: A) T4: soybean oil + alpha-lipoic acid; B) T5: sesame oil + linseed oil + alpha-lipoic acid; C) T6: linseed oil + alpha-lipoic acid, evidencing hepatocytes (black arrow), cordonal arrangement of hepatocytes (red arrow), sinusoids (yellow arrow), vascular congestion (pink arrow) and portal vein (green arrow).

DISCUSSION

Growth performance

In the biometry performed at the 90th day, a high heterogeneity was observed among fish weight within each experimental unit. The heterogeneity observed in the pacu juveniles may be due to the hierarchical relationship between the animals, where the larger animals consumed more than the smaller ones.

In a study with pacu consuming diets containing 22 and 25 % CP, IWGs (39.9 to 57.8 g) were similar to those obtained in this study (ABIMORAD and CARNEIRO, 2007). Thus, it is possible to verify that lower CP levels in pacu diets can promote weight gain approximately equal to that obtained with diets with higher protein levels, and may suggest lower protein requirements for this species.

In some studies with a similar duration time, higher values of diet intake were observed, such

as in FERNANDES *et al.* (2001), where feed intake varied from 151.6 to 157.9 g, and in MUÑOZ-RAMÍREZ and CARNEIRO (2008), where it ranged from 104.4 to 129.6 g.

The results of feed conversion ratio in this study were similar to the values found by MUÑOZ-RAMÍREZ and CARNEIRO (2008), with results varying between 1.33 and 1.58. However, FCR values obtained for pacu were better than those found by FERNANDES *et al.* (2001), who obtained FCR ranging from 3.34 to 4.14; ABIMORAD and CARNEIRO (2007), whose FCR values were 2.7 to 3.0; SANTOS *et al.* (2009), with results between 3.07 and 3.60, even using diets with 28% CP; and by SIGNOR *et al.* (2010), who found FCR from 2.88 to 3.16 in pacu juveniles fed diets containing higher protein levels (25, 30 and 35%) than the levels adopted in the present study.

Lower indexes of protein efficiency ratio were verified by FERNANDES *et al.* (2001), with PERs of 1.29 to 1.44%; ABIMORAD and CARNEIRO

(2007), where it varied from 1.5 to 1.6%; and SANTOS *et al.* (2009), with values ranging from 1.00 to 1.21%.

The results of specific growth rate were better than those obtained by FERNANDES *et al.* (2001), that ranged from 0.31 to 0.34%; ABIMORAD and CARNEIRO (2007), between 0.76 and 0.80%; MUÑOZ-RAMÍREZ and CARNEIRO (2008), with SGRs of 1.76 to 2.27%; and SIGNOR *et al.* (2010), whose values varied from 0.67 to 0.70%, even with diets with higher CP levels (25, 30 and 35 %).

Fish fed diets with LO (T3 and T6) showed a better average final weight, while those fed diets with SBO presented the lower final weight. Individual weight gain followed the same trend, with better results to the fishes fed diets T3 and T6.

The presence or absence of alpha-lipoic acid in the diets had no effect ($P>0.05$) on the mean final weight of the animals. However, in a study with pacu juveniles fed diets containing ALA (100 mg 100 g⁻¹) and ascorbic acid (50 mg 100 g⁻¹), there was an influence ($P<0.05$) of this compound on the final weight of the fish. Fish consuming diets with ALA and without ascorbic acid had a lower average weight (51.7 g), but for the treatments with ALA associated with ascorbic acid the index was improved (58.6 g) (TRATTNER *et al.*, 2007).

Chemical composition of pacu fillets

The experimental treatments had no effect ($P>0.05$) on the chemical composition of the fillets. When feeding pacus with diets containing different CP levels (18.5 to 28.3 %), KLEIN *et al.* (2014) observed that the diets with lower CP content decreased the humidity (69.04%) and increased the ether extract (11.88%) of fish fillets, whereas diets with higher CP content had an opposite effect, resulting in fillets with higher humidity (72.65%) and lower ether extract (8.00%).

The source of oil used in the diets did not influence ($P>0.05$) the CP levels of the fillets, but the ALA caused a difference ($P<0.05$) between the means of CP. It was observed that fish fed diets without ALA presented fillets with higher CP levels.

Higher levels of CP (18.89%) were found in wild pacu fillets (RAMOS FILHO *et al.*, 2008) and

in fillets of pacu fed diets containing 27.98% CP and 3.25% total lipids, ranging from 18.62 to 18.79% (SANTOS *et al.*, 2009).

Liver histology

The livers from pacu juveniles were light brown in color, typical of herbivorous fish (BERNET *et al.*, 2004). The shape and constitution of the liver observed were the same as those described for several teleostean fish species studied (HINTON *et al.*, 1972; HINTON and POOL, 1976; ROCHA *et al.*, 1994; BRUSLÉ and ANADON, 1996; ROCHA *et al.*, 1997; VICENTINI *et al.*, 2005; FUJIMOTO *et al.*, 2008), with no particular characteristics in the treatments.

Although the liver's structure varies according to species, sex, age, diet or even environmental conditions (GENTEN *et al.*, 2009), some changes observed through histological analysis may be indicative of animal stress or inadequate nutrition.

CHEN *et al.* (2013) evaluated the effects of diets containing different levels (0.00, 0.10, 0.32, 0.63, 0.98, 1.56 and 2.04 % of dry weight) of linolenic acid (LNA, 18:3n-3) for Nile tilapia (*Oreochromis niloticus*) and observed that fish fed diets with 0.10% of LNA increased the lipid content stored in hepatic tissue and showed the highest hepatosomatic index.

In this study, histological analysis of the livers of the fish that received the diet supplemented with alpha-lipoic acid (T4, T5 and T6), indicated the presence of blood congestion at some points. However, it is important to point out that better health conditions for animals are not characterized by the complete absence of histopathological alterations; so it is normal to verify some changes, such as mild inflammatory reactions, small structural modifications (BERNET *et al.*, 2004; BARBIERI and BONDIOLI, 2103) or even the appearance of moderate blood congestion, as observed in the present study.

The nucleus of the hepatocytes in all groups of fish analyzed was centralized, indicating that the cell had normal metabolic activity. The presence of cytoplasmic vacuolations increases the volume of hepatocytes, displacing the nucleus to the periphery of the cell, and indicates the existence of regions with a probable concentration

of lipids and glycogen, or the combination of toxic agents with intracytoplasmic lipids (SANTOS *et al.*, 2004).

It is important to note that the diet may influence the structure of the liver and can, depending on the quality of the food, cause lesions in the hepatic tissue (ROCHA *et al.*, 2010). It was evidenced that the experimental diets in this study did not cause major alterations in the liver histology of fish fed diets with different lipid sources and levels of alpha-lipoic acid.

In cases of inadequate nutrition, it is possible to observe some characteristics in liver histology such as alterations of basic structures, with nuclei located in the hepatocyte peripheries, cytoplasmic vacuolization and cell necrosis (RAŠKOVIĆ *et al.*, 2011). As no significant changes were observed in pacu livers, it can be stated that the experimental diets were nutritionally adequate, besides not causing damage to fish health.

CONCLUSIONS

Linseed oil improves final body weight and individual weight gain of pacu juveniles. The addition of alpha-lipoic acid in the diets does not alter the growth performance of the animals, and, in the absence of this acid, pacu fillets accumulate higher levels of crude protein. None of the vegetable oil sources (soy, sesame and linseed) evaluated caused changes to pacu liver cells, although the addition of alpha-lipoic acid promoted minor changes.

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