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SOURCES OF LIPIDS IN DIETS FOR SILVER CATFISH (*Rhamdia quelen*) JUVENILES

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ABSTRACT

The purpose of this study was to evaluate different lipid sources in diets for silver catfish juveniles regarding aspects of productive performance, centesimal composition, hepatic histology, biochemical responses, and oxidative stress. A total of 300 juveniles with an initial mean weight of 18.45 ± 1.22g were distributed in 20 net-pens of 1m^3, arranged in a 200 m^2 masonry tank, for a period of 90 days. The experimental diets were isoprotein (29.05% digestible protein) and isoenergetic (3,250 kcal.kg-1), containing different sources of oils (soybean, sunflower, fish, canola and olive) at a concentration of 3.0%. The animals were distributed in a completely randomized design, with five treatments and four replicates. The data were submitted to ANOVA, and the means, when significant, were compared by the Tukey test at 5% significance level. Visceral fat index was higher for fish fed diets containing sunflower oil (P<0.05). No differences (P>0.05) were observed for the variables of productive performance, centesimal composition, and blood biochemistry. A difference (P<0.05) was observed in hepatic histology, where the treatment with soybean oil had a higher number of hepatocytes. The animals fed with fish oil had a higher TBARS lipid peroxidation and a higher GST enzyme activity (P<0.05). Therefore, the different lipid sources can be used to feed this species without any damage to productive performance. It is not advisable to include sunflower oil, as it provides greater deposition of visceral fat.

Key words: fatty acids; aquaculture; fish nutrition; *Rhamdia quelen*.

FONTES DE LIPÍDIOS EM DIETAS PARA JUVENIS DE JUNDIÁ (*Rhamdia quelen*)

RESUMO

O objetivo do presente estudo foi avaliar diferentes fontes lipídicas em dietas para juvenis de jundiá sobre o aspecto do desempenho produtivo, composição centesimal, histologia hepática, respostas bioquímicas e estresse oxidativo. Foram utilizados 300 juvenis com peso médio inicial de 18,45 ± 1,22g, distribuídos em 20 tanques-rede de 1m^3, dispostos em um tanque de alvenaria de 200 m^2, por um período de 90 dias. As dietas experimentais foram isoproteicas (29,05% de proteína digestível) e isoenergéticas (3.250 kcal.kg^{-1}), contendo diferentes fontes de óleos em sua composição: soja, girassol, peixe, canola e oliva na concentração de 3,0%. Os animais foram distribuídos em um delineamento inteiramente aleatório, com cinco tratamentos e quatro repetições. Os dados foram submetidos a ANOVA, e quando significativa as médias foram comparadas por Tukey a 5% de significância. O índice de gordura visceral foi maior para os peixes alimentados com dietas contendo óleo de girassol (P<0,05). Não foram observadas diferenças (P>0,05) para as variáveis de desempenho produtivo, composição centesimal e bioquímica sanguínea. Foi observado diferença (P<0,05) na histologia hepática, onde o tratamento com óleo de soja apresentou maior número de hepatócitos. Os animais alimentados com óleo de peixe apresentaram uma maior peroxidação lipídica TBARS e maior atividade da enzima GST (P<0,05). Portanto, as diferentes fontes lipídicas podem ser utilizadas na alimentação desta espécie sem que ocorram prejuízos sobre o desempenho produtivo. Não se recomenda a inclusão de óleo de girassol, devido ao mesmo proporcionar uma maior deposição de gordura visceral.

Palavras-chave: ácidos graxos; aquicultura; nutrição de peixes; *Rhamdia quelen*.

INTRODUCTION

Brazil has an enormous water availability, which makes it a promising country in the production of aquatic organisms. Over the past few years, the country has presented significant growth in continental fish farming (FAO, 2018), and the cultivation of

native species increased considerably (IBGE, 2016). However, there is still a lack for studies focused on management techniques and production of these species in captivity, in order to boost the productive chain of freshwater fish in Brazil and worldwide.

In the southern region of the country, *Rhamdia quelen*, popularly known as Silver catfish, stands out due to its characteristics of rapid growth in low temperature environments, resistance to handling, easy adaptation to environmental variables and intensive cultivation, easy reproduction, omnivorous eating habits with good use of the most varied foods, in addition to the good acceptance of its meat by the consumer market (Carneiro et al., 2003; Fracalossi et al., 2004; Parra et al., 2008; Signor et al., 2013; Feiden et al., 2010; Diemer et al., 2012).

Nutrition is a limiting factor for fish growth. The development of highly balanced diets to meet the nutritional requirements of animals in order to maximize their productive performance is paramount. In fish feeds, protein is the most expensive nutrient in diet formulation (Wilson, 2002). Additionally, protein is used by aquatic organisms (in particular freshwater fish) as sources of energy (Sargent et al., 2002). Nevertheless, the inclusion of other nutrients in diets to serve as energy sources is interesting at the nutritional and functional level, in order to contribute to the reduction of the use of protein as energy in order to intend it exclusively for animal growth (Shiau, 2002; Martino et al., 2002). In this case, lipids may be an alternative, as this nutrient provides twice as much energy as proteins and carbohydrates through the oxidation process (Lehninger et al., 2011).

Lipids are organic molecules used as sources of energy and essential fatty acids in animal nutrition (Bertechini, 2013). They also act as carriers of fat-soluble vitamins, in addition to performing important functions in biochemical and hormonal processes, maintaining the permeability and flexibility of cell membranes (Haliloğlu et al., 2004; Leonard et al., 2004).

There are a variety of lipid sources providing essential fatty acids of both animal and vegetable origin used as ingredients for fish diets. Fish oil is a lipid source of animal origin, whose chemical composition consists of approximately of 0.6-10.5% linoleic acid (C18:2 n-6); of 0.4-2.5% linolenic acid (C18: 3 n-3); of 0.1-1.6% arachidonic acid (C20: 4 n-6), 0.4-17.4% eicosapentaenoic acid (C20: 5 n-3); and 1.3-23.5% docosahexaenoic acid (C22: 6 n-3) (NRC, 2011).

Soybean oil is a lipid source of vegetable origin, consisting of 51% n-6 linoleic acid and 6.8% n-3 linolenic acid. Sunflower oil has 65.7% of n-6 linoleic acid in its chemical composition, however, it does not contain n-3 linolenic acid. Canola oil contains 20.2% n-6 linoleic acid and 12% n-3 linolenic acid. Olive oil has, in its chemical composition, 7.9% linoleic acid in the n-6 series and 0.6% linolenic acid in the n-3 series (NRC, 2011).

The adequate supply of animal and vegetable oil supplementation can enhance growth without impairing the physiological responses of the animals. This study aimed to evaluate the effects of supplementation of different lipid sources on the productive performance, centesimal composition, hepatic histology, biochemical parameters and enzymatic activity in silver catfish juveniles.

MATERIAL AND METHODS

Place and period

The experiment was conducted at the Environmental Aquaculture Research Institute (InPAA), Western Paraná State University (Unioeste), Toledo, State of Paraná, Brazil. The experimental period was 90 days (October to January 2016).

This study was approved by the Committee on Ethics in the Use of Animals (CEUA) of the same institution under protocol Nº. 08/17.

Animals and experimental design

A total of 300 silver catfish (*Rhamdia quelen*) juveniles with an initial mean weight of 18.45 ± 1.22 g were distributed in 20 net-pens with 1 m^3 in volume, arranged in a 200 m^2 masonry tank. The experimental design was completely randomized with five treatments and four replicates, totaling 15 fish per experimental unit.

Water quality

The physical-chemical variables were monitored during the experimental period, with weekly measurements of dissolved oxygen (6.0 ± 0.8 $mg.L^{-1}$), pH (6.7 ± 0.2) and electrical conductivity (36.1 ± 1.5 $\mu S.cm^{-1}$), with the aid of a multiparameter probe (YSI, Pro Plus, Yellow Springs-Ohio, United States). The water temperature (23.6 ± 2.1 °C) was measured daily in the morning and afternoon by means of a digital thermometer. The parameters of water quality remained in the ideal range for the productive performance of the species (Baldisserotto and Radünz Neto, 2004).

Experimental diets and food management

Five isoenergetic (3250 kcal ED/kg feed) and isoprotein (29.09% PD) diets were prepared. The diets contained different oil sources in their composition (soybean, sunflower, fish, canola, and olive) in the concentration of 3.0% (Table 1). The ingredients were ground in a hammer mill with a 0.6 mm mesh sieve, mixed and extruded (Ex-Micro® extruder, Ex-Micro, Ribeirão Preto – São Paulo, Brazil). Simultaneously, the feeds were subjected to forced ventilation drying (55 °C) during the 24-hour period. The feeds then received the necessary oils manually by spraying and were mixed and stored in a freezer during the experimental period. The fish were fed four times a day (8:30 am, 11:00 am, 2:00 pm, and 5:30 pm) to apparent satiation.

Data collection and productive performance

At the end of the experiment, the fish were fasted for 24 hours to empty the gastrointestinal tract and subsequently desensitized with a solution containing 100 $mg.L^{-1}$ benzocaine for individual measurements of weight (g) and length (cm) and blood tests. Three fish from each experimental unit were euthanized in benzocaine, at a dose of 250 $mg.L^{-1}$ (Gomes et al., 2001), and then stored on ice for removal of visceral fat and liver.

The productive performance data evaluated were weight gain (g) (final body weight – initial body weight); survival (%) [(final number of fish / initial number of fish)] * 100; apparent feed conversion (diet consumed / weight gain); specific growth rate (% day^{-1}) [(ln (final weight) – ln (initial weight)) / days of experiment] * 100; protein efficiency ratio [weight gain (g) / crude protein consumption in dry matter (g)]; hepatosomatic index (%) [liver weight (g) * 100 / final weight (g)]; and visceral fat (%) [visceral fat weight (g) * 100 / final weight (g).

Centesimal composition analysis

The centesimal composition of the fish was determined by the methodology proposed by AOAC (2005), being: humidity (pre-drying in forced air ventilation oven at 55 °C for 72 hours, followed by drying at 105 °C for 8 hours); proteins (Kjeldhal method; Modle MA-036, Piracicaba - São Paulo, Brazil); ethereal extract (Soxhlet extractor with petroleum ether as solvent; Modle TE-0,44, Piracicaba - São Paulo, Brazil); and mineral matter

Table 1. Percentage and centesimal composition of the experimental feeds containing different oils, for silver catfish (*Rhamdia quelen*) juveniles.

Ingredients (%)	Lipid sources				
	Soybean oil	Sunflower oil	Fish oil	Canola oil	Olive oil
Fish flour	33.30	33.30	33.30	33.30	33.30
Corn grain	18.89	18.89	18.89	18.89	18.89
Rice flour	12.65	12.65	12.65	12.65	12.65
Poultry offal flour	12.55	12.55	12.55	12.55	12.55
Soybean meal 45%	10.00	10.00	10.00	10.00	10.00
Wheat meal	8.00	8.00	8.00	8.00	8.00
Soybean oil	3.00	-	-	-	-
Sunflower oil	-	3.00	-	-	-
Fish oil (tilapia)	-	-	3.00	-	-
Canola oil	-	-	-	3.00	-
Olive oil	-	-	-	-	3.00
Premix[1]	1.00	1.00	1.00	1.00	1.00
Table salt	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.10	0.10	0.10	0.10	0.10
Antifungal	0.30	0.30	0.30	0.30	0.10
Vitamin C	0.10	0.10	0.10	0.10	0.10
Antioxidant (BHT)	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100
Nutrients (%)					
Digestible protein	29.09	29.09	29.09	29.09	29.09
Linoleic acid	2.97	2.97	2.97	2.97	2.97
Starch	25.00	25.00	25.00	25.00	25.00
Total arginine	2.67	2.67	2.67	2.67	2.67
Calcium	3.21	3.21	3.21	3.21	3.21
Digestible energy (kcal)	3250	3250	3250	3250	3250
Crude fiber	1.68	1.68	1.68	1.68	1.68
Total phosphorus	1.45	1.45	1.45	1.45	1.45
Fat	9.36	9.36	9.36	9.36	9.36
Total lysine	2.01	2.01	2.01	2.01	2.01
Total methionine	0.71	0.71	0.71	0.71	0.71
Chemical composition (natural matter %)					
Gross energy (Kcal)	4540.00	4480.00	4500.00	4480.00	4480.00
Crude protein	38.43	39.06	39.08	38.28	39.47
Fat	8.60	8.03	8.68	8.79	8.84
Mineral matter	3.84	4.05	3.64	4.23	4.05

[1] Guarantee levels per kilogram of product: vit. A = 500,000 IU; vit. D3 = 250,000 IU; 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; calcium pantothenate = 4,000 mg; vit. C = 10,000 mg; biotin = 10 mg; Inositol = 1,000; nicotinamide = 7,000; choline = 10,000 mg; Cobalt = 10 mg; Copper = 1,000 mg; Iron = 5,000 mg; Iodine = 200 mg; Manganese = 1,500 mg; Selenium = 30 mg; Zinc = 9,000 mg.

(calcination of samples at 550 °C for 6 hours, Modle 2000B, Belo Horizonte - Minas Gerais, Brazil).

Liver histology

For hepatic histological assessment, the livers of three animals per experimental unit were collected to determine the number of hepatocytes, totaling 12 fish per treatment. These samples were fixed in an Alfac solution for 24 hours and then transferred to flasks containing 70° alcohol. The material was dehydrated by passages in increasing series of alcohols, diaphanized in xylol and embedded in paraffin, to obtain serial cross sections (7µm in thickness) with the aid of a rotating microtome (Thermo Scientific – Microm HM 340E). To determine the number of hepatocytes per area (counting area: 2,000µm^2), the slides were stained with Hematoxylin and Eosin (HE), as described by Bancroft and Stevens (1982).

The photodocumentation (image capture) was obtained by means of an optical microscope (P1 Olympus BX 50 – Manila, Philippines) coupled to a camera (Olympus PMC 35 B – Berlin, Germany), using a 40x, in 8 images per cut (24 images per animal), totaling 192 images per treatment. Morphological alterations were evaluated qualitatively through a lesion index calculated according to Bernet et al. (1999).

The changes were classified into three factors of importance (w): (1) reasonable injury; (2) moderate injury; and (3) irreversible injury, which leads to partial or total organ loss. Each histopathological change was evaluated by using scores (α) that varied from 0 to 6, depending on the degree of alteration, being (0) without change; (2) low occurrence; (4) moderate occurrence; (6) serious injury. To determine the lesions, we used the Bernet index = Σ factor of importance (w) x score (α). A table was developed for the present study (Table 2), with the main histopathological lesions found in the study. These measurements were performed with the aid of an image analysis system (cellSens Standard 1.15® software program).

Blood tests

For the evaluation of the blood parameters, an aliquot of blood was collected in three fish from each experimental unit through the caudal puncture, with the aid of a heparinized syringe. Subsequently, biochemical analyses of total protein (g.dL^{-1}), total cholesterol (mg.dL^{-1}), glucose (mg.dL^{-1}) and triglycerides (mg.dL^{-1}) were performed. To obtain the plasma, the samples were centrifuged at 2,500 rpm for five minutes. Commercial kits (Gold Analisa®) were used for the analysis, the reading being processed in a spectrophotometer, according to the manufacturer's instructions.

Table 2. Changes found in the liver of silver catfish (*Rhamdia quelen*) juveniles fed diets containing animal and vegetable oils.

	Bernet index	
	Changes observed in the liver	**Factor of importance**
Hepatocyte	IFL	2
	MM	1
	N	3
	CS	1
	LLC	2
	VC	1

IFL = Leukocyte inflammation; MM = Melanomacrophagous; N = Necrosis; CS = Congestion of sinusoids; LLC = Loss of cell limit; VC = Cytoplasmic vacuolization.

Oxidative stress

At the end of the experimental period, analyses were performed on thiobarbituric acid reactive substances (TBARS), Glutathione-S-transferase (GST), and catalase (CAT). Two fish from each experimental unit, packed in ice, were used. A liver sample was then removed and stored in liquid nitrogen.

The liver samples were homogenized in 9 mL of 0.3M sodium phosphate buffer (140mM KCl, pH 7.4). They were then centrifuged at 10,000 x g for 10 minutes at 4 °C. The liver supernatant was used to perform the TBARS, GST and CAT analyses.

For the determination of TBARS, the methodology described by Buege and Aust (1978) was used, and the results were expressed in n mol MDA/mg protein. The catalase activity (CAT) was determined according to Nelson and Kiesow (1972) at a wavelength of 240 nm, and the results were expressed as µmol/mg protein/min. In turn, the Glutathione S-transferase (GST) activity was measured at 412 nm in a spectrophotometer according to the Ellman method (1959) using 20% Trichloroacetic Acid (20% TCA = 20g TCA in 100 mL distilled water) in the proportion of 0.2 g of tissue per 1 mL of TCA. The reading was performed in a spectrophotometer at a wavelength of 412 nm, and the activity was expressed in µmol.mg^{-1} protein.

Statistical analysis

The data were submitted to the analysis of normality and homoscedasticity and, considering these assumptions, were submitted to analysis of variance (ANOVA). When they presented significant differences, a comparison test was performed by the Tukey means, at 5% significance level. The Bernet index was submitted to non-parametric Kruskal-Wallis statistics. The analyses were performed by Statistic 7.1 software®.

RESULTS

The silver catfish fed a diet containing animal and vegetable oils presented similar results (P>0.05) for weight gain, survival, apparent feed conversion, specific growth rate, protein efficiency ratio and hepatosomatic index. Meanwhile, the visceral fat index was higher (P<0.05) in fish fed diets containing sunflower oil (Table 3).

The centesimal composition of the fish did not differ (P>0.05) between diets containing animal and vegetable oils (Table 4).

Regarding hepatic histology (Table 5), the amount of liver cells was higher for the treatment containing soybean oil (P <0.05).

No significant difference was found between the treatments for the lesion index in the liver (Figure 1). In the present study, the

Table 3. Productive performance of silver catfish (*Rhamdia quelen*) juveniles fed diets containing animal and vegetable oils.

Variables*	Oils						
	Soybean	Sunflower	Fish	Canola	Olive	CV	P
WG (g)	104.83	106.12	100.84	106.62	102.21	9.13	NS
SO (%)	98.33	100	93.33	95	95	3.36	NS
AFC	1.38	1.37	1.53	1.42	1.4	11.19	NS
SPR (%/day)	2.28	2.28	2.03	2.2	2.16	4.37	NS
PER	2.07	2.08	1.97	2.14	2	11.81	NS
HSR (%)	2.21	2.07	2.44	2.2	2.28	16.54	NS
VSFI (%)	2.24a	3.26b	2.38a	2.35a	2.42a	18.19	0,01

WG = Weight Gain (g); SO = Survival (%); AFC = Apparent Food Conversion; SPR = Specific Growth Rate (% / day); PER = Protein Efficiency Rate; HSR = Hepatosomatic Rate (%); VSFI = Visceral Fat Index (%). *Values followed by distinct letters on the same line differ statistically by the Tukey test (P<0.05). CV = Coefficient of variation.

Table 4. Centesimal composition of silver catfish (*Rhamdia quelen*) juveniles fed diets containing animal and vegetable oil.

Variables*	Oils						
	Soybean	Sunflower	Fish	Canola	Olive	CV	P
HU (%)	71.32	70.65	70.93	72.76	71. 06	2.28	NS
CP (%)	15.8	16.03	15.72	18.17	16.33	8.41	NS
EE (%)	10.3	10.03	10.07	9.45	9.64	10.39	NS
MM (%)	3.22	3.48	3.34	2.93	3.57	9.39	NS

HU = Humidity (%); CP = Crude Protein (%); EE = Ethereal Extract (%); MM = Mineral Matter (%); CV = Coefficient of variation; *Not significant (P>0.05). *Not significant (NS). Probability of significance (P).

Table 5. Hepatic histology of silver catfish (*Rhamdia quelen*) juveniles fed diets containing animal and vegetable oil.

Variables*	Oils						
	Soybean	Sunflower	Fish	Canola	Olive	CV	P
HE**	172b	115.91a	146.08ab	131.69a	123.70a	17.02	0.02

HE = Hepatocytes; CV = Coefficient of variation; **Counting area: 2,000 μm^2; *Values followed by distinct letters on the same line differ statistically by the Tukey test (P<0.05). *Not significant (NS). Probability of significance (P).

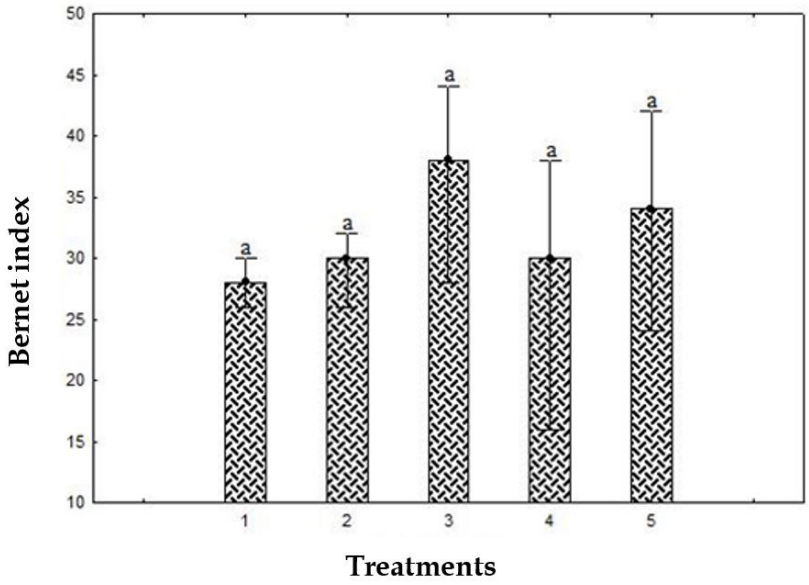

Figure 1. Index of histophatological lesions of silver catfish (*Rhamdia quelen*) juvenilies fed diets containing animal and vegetable oil. Values followed by similar letters no differ statistically by the Tukey test (P>0.05).

following changes in the liver of the fish were observed: leukocyte inflammation, melanomacrophages, sinusoid congestion, loss of cell limit and cytoplasmic vacuolization (Figures 2A and 2B).

In the evaluation of plasma biochemistry of silver catfish (Table 6), there were no significant differences (P> 0.05).

In relation to oxidative stress (Table 7), the variables evaluated in the liver TBARS and GST presented a significant difference for the treatment containing the inclusion of fish oil (P <0.05). The activity of the CAT enzyme in this same organ showed no significant difference between diets containing different sources of animal and vegetable oils (P> 0.05).

DISCUSSION

Experimental diets presented constant energy nutritional values at the base of the feed and were elaborated to meet the nutritional requirements of the species, and, in this way, the fish were probably able to efficiently harness the energy and fatty acids of the evaluated oils. This fact may explain the similar responses of the productive performance of the animals fed with different lipid sources. Similarly, Losekann et al. (2008), evaluating juveniles of silver catfish fed with different sources of vegetable oils, verified that there were no significant differences in the zootechnical performance of the species. Similar results were found by Melo et al. (2002) when they tested different lipid sources for the same species.

In this current study, visceral fat index was higher in fish fed diets containing sunflower oil. This difference, however, did not reflect higher growth and weight of the animals. This increase may possibly be related to the chemical composition of the present oil, which was above the capacity of the silver catfish to use as energy in this phase of life, favoring the storage as visceral fat. The accumulation of visceral fat directly affects the reduction of the fillet yield, the palatability of the meat, causing the rejection of the consumer market (Meurer et al., 2002).

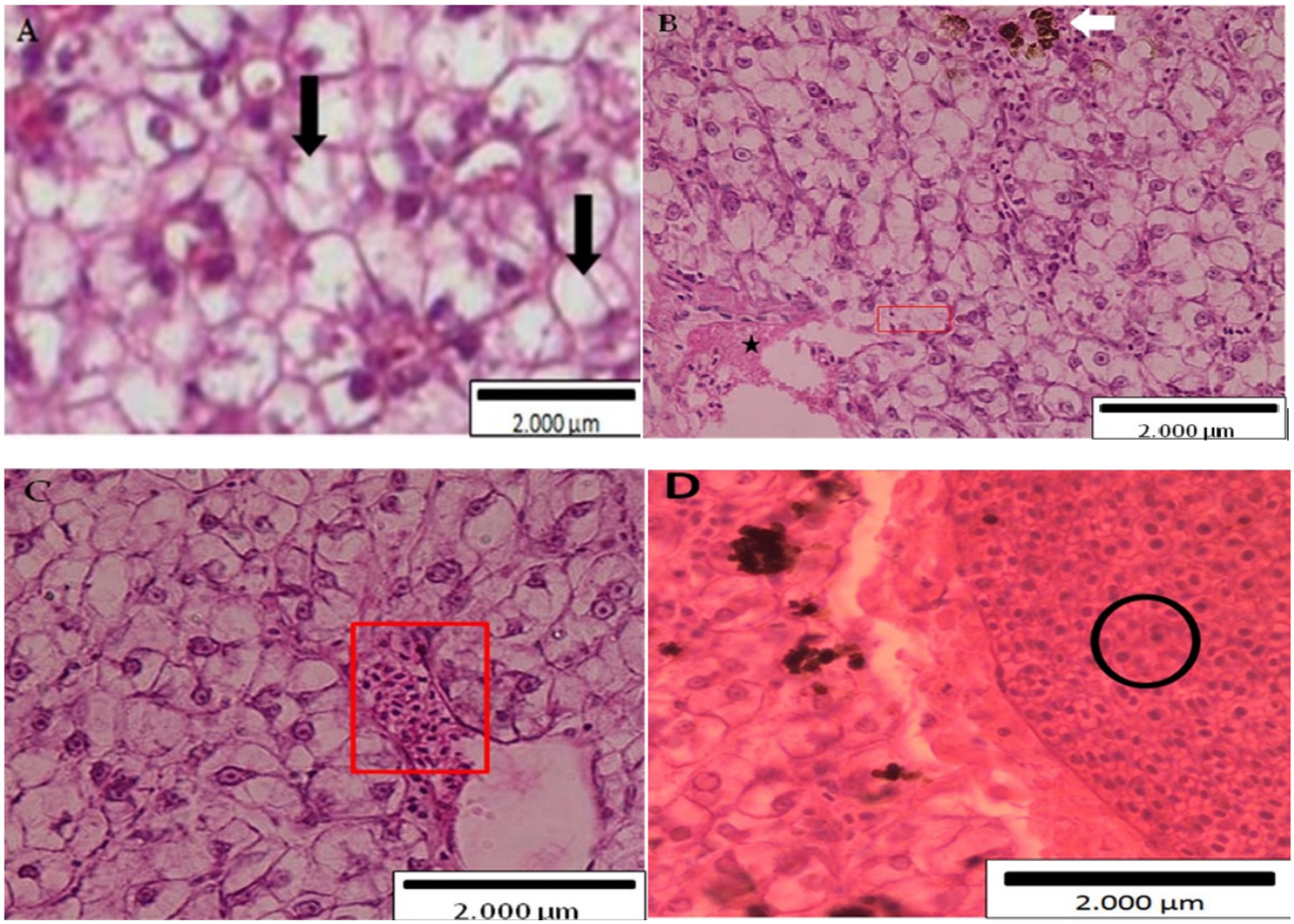

Figure 2. Hepatic histological section of silver catfish (*Rhamdia quelen*) juveniles (HE, 40x objective). (A) Cytoplasmic vacuoles characteristic of hepatic steatosis (black arrow); (B) Melanomacrophage (white arrow); Loss of cell limit (rectangle area); Necrosis area (star); (C) Congenstion of sinusoids (red square); (D) Leukocyte infiltration (circle area).

Table 6. Plasma biochemical parameters of silver catfish (*Rhamdia quelen*) juveniles fed diets containing animal and vegetable oil.

Variables*	Oils						
	Soybean	Sunflower	Fish	Canola	Olive	CV	P
TRIG	533.23	492.77	518.6	504.52	383.78	27.62	NS
CHOL	332.68	368.25	332.16	354.22	324.47	20.98	NS
PROT	4.91	4.83	5.45	5.24	6.08	19.72	NS
GLUC	78.53	88.41	102.07	68.46	96.95	32.28	NS

TRIG = Triglycerides (mg.dL^{-1}); CHOL = Total cholesterol (mg.dL^{-1}); PROT = Total proteins (g.dL^{-1}); GLUC = Glucose (mg.dL^{-1}); CV = Coefficient of variation; *Not significant (P>0.05). *Not significant (NS). Probability of significance (P).

Table 7. Oxidative stress analysis in silver catfish (*Rhamdia quelen*) juveniles fed diets containing lipid sources of animal and vegetable origin.

Variables*	Oils						
	Soybean	Sunflower	Fish	Canola	Olive	CV	P
TBARS*	3.36bc	2.75ab	4.27c	2.20ab	1.77a	20.90	0.01
GST*	0.73a	0.91ab	1.08b	0.94ab	0.74a	11.28	0.02
CAT	0.68	0.82	0.72	0.39	0.6	38.62	NS

TBARS = thiobarbituric acid reactive substances (n mol MDA / mg protein); GST = Glutathione S-Transferase (µmol / mg protein); CAT = Catalase (µmol / mg protein / min); CV = Coefficient of variation; *Values followed by distinct letters on the same line differ statistically by the Tukey test (P<0.05). Probability of significance (P).

Differently, Neu et al. (2013) testing different glycerol concentrations in diets for tilapia (25 g kg^{-1}, 50 g kg^{-1}, 75 g kg^{-1} and 100 g kg^{-1}) and a control diet, verified that there were no differences for visceral fat index, corroborating with the results obtained by Lui et al. (2017) who evaluated the influence of different fish oil acidity levels(0.24, 1.48, 6.40 and 9.85), with the inclusion of 3.5% in diets for tilapia fingerlings.

The centesimal composition of the fish did not differ between treatments. The animals, when fed with isoprotein and isoenergetic diets containing the same levels of lipid inclusion generally do not present differences in the basic chemical composition. Furthermore, macronutrients play an energetic role in the animal metabolism, serving their nutritional requirements (Menoyo et al. 2003; Lehninger et al., 2011).

The liver of teleosts is responsible for several vital functions in the animal, being formed by hepatocytes, mitotic cells (Costa et al., 2012). Its function is related to protein, lipids and carbohydrates synthesis, storage of some nutrients, in addition to having the capacity to biotransform xenobionts produced by the animal (Bruslé and Anadon, 1996).

Regarding the liver analysis, the fish that received the diet containing soybean oil had a higher number of hepatocytes. Possibly, the greater amount of liver cells suggests that the oil promotes a hepatoprotective action in order to reduce its own oxidation, thus avoiding substances harmful to fish health (Zelikoff, 1998).

Although there was no difference between the treatments in the liver morphology, histopathological changes observed maybe related to the food being supplied until the apparent satiety, constantly overloading and demanding the synthesis and breakdown of these molecules as well as causing the appearance of vacuolization of the hepatocytes.

The deposition of lipids in fish hepatocytes with increased energy levels was observed by Bombardelli et al. (2009) when evaluating different levels of digestible energy in diets for Nile tilapia (*Oreochromis niloticus*) females. Bolla et al. (2011) tested the effect of three diets (1st natural food, 2nd commercial ration and 3rd commercial ration supplemented with herring) for Atlantic halibut (*Hippoglossus hippoglossus*), and reported that females fed the diet containing commercial ration and herring presented cytoplasmic vacuoles.

Hepatic steatosis occurs when the amount of energy supplied exceeds the liver's ability to oxidize fatty acids, favoring the storage of fat in liver tissue in the form of triglycerides (Li et al., 2014; Rezende et al., 2018). This accumulation of energy occurs through increased activity of the enzymes in the process of lipogenesis. In this way, nutrition plays an important role in the metabolism of fish, as it may compromise animal health and organ function as the liver through lipid increase (Caballero et al., 1999) and unbalance of fatty acids (Caballero et al., 2004). Thus, to improve and optimize good nutrition for fish, it is essential to have information about the species' eating habits and the ingredients to be used for a better accuracy of balanced diets.

Analyzes of biochemical parameters of the blood can aid in the evaluation of animal health (Satake et al., 2009; Bosisio et al., 2017). However, there is little information in the literature regarding these parameters, due to the great diversity of cultivated species, different breeding systems, life stages, sex, feeding habits and fish blood behavior in relation to the diets supplied (Soldatov, 2005). Nevertheless, Borges et al. (2004), in a study with silver catfish (*Rhamdia quelen*), in a closed system with boxes of 500L

and fed with commercial feed, reported blood concentrations of the values in the interval of 3.5-4.9 ($g.dL^{-1}$) for total protein, 43-78 ($mg.dL^{-1}$) for glucose, 138-546 ($mg.dL^{-1}$) for triglycerides, and 110-240 ($mg.dL^{-1}$) for total cholesterol.

Cholesterol is present in the cell membrane of the body, being the main precursor of hormones and vitamin D, and is an essential substance for the body in the performance, reproduction, and homeostasis of animals (Caula et al., 2008). In this study, higher values were verified than those proposed by Borges et al. (2004). Possibly, these high cholesterol levels may be related to the reproductive period of the species, which led the liver to synthesize the steroids by increasing cholesterol in the bloodstream of the fish.

Plasma proteins are composed of albumins, globulins and fibrinogen and are used to evaluate the nutritional status of the organism. In this study, lipid sources did not promote changes in plasma protein concentrations for silver catfish catfish juveniles. Similar results were observed by Higuchi et al. (2011), in which different protein levels (25%, 30% and 35% crude protein) and two levels of digestible energy (3,250 and 3,500 kcal) did not influence plasma protein levels for the same species. The values obtained from the respective studies are close to the reference range proposed by Borges et al. (2004), considered normal for healthy silver catfish.

The plasma glucose concentration in this study presented changes above what is used as a reference for the species and may be related to harvest management, allowing the fish to use glucose as the main source of energy to overcome stress situations (Tavares-Dias and Moraes, 2010; Barbieri et al., 2016).

In the present study, a significant increase of TBARS was observed in the liver of the silver catfish fed with fish oil containing diets, through a higher presence of malonaldehyde. The increase of TBARS in the liver indicates that the inclusion of fish oil in silver catfishdiets resulted in greater lipid peroxidation in its tissue, consequently the reduction of the antioxidant capacity of this lipid source in the production of reactive oxygen molecules, implying oxidative stress. Ng et al. (2013) reported a higher concentration of TBARS in the fillet of hybrid tilapia fed with diet containing fish oil. Possibly, these results can be explained by the chemical composition of this oil that presents high amount of polyunsaturated fatty acids, susceptible to oxidative damages.

The diet containing fish oil resulted in a greater activity of the GST enzyme for the fish of this study. This increase may represent the antioxidant capacity of this enzyme in the removal of foreign chemical compounds present in the peroxidation, as this antioxidant enzyme plays the role of detoxifying the reactive oxygen species present in the organism (Salbego et al., 2017).

In this study, the catalase enzymatic activity did not present statistical difference between the treatments, due to the non-release of hydrogen peroxide by the process of β-oxidation. Catalase converts hydrogen peroxide into oxygen and water and, because it is an intracellular enzyme located in peroxisomes, has the ability to degrade fatty acids through β-oxidation (Nelson and Cox, 2005; Motta, 2011).

CONCLUSION

Supplementation with different lipid sources of animal and vegetable origin provides similar performance for silver catfish (*Rhamdia quelen*) juveniles. Sunflower oil is not recommended, as it provides a greater deposition of visceral fat in the fish. In addition, supplements with appropriate lipid sources induce the appearance of cytoplasmic vacuoles in hepatocytes, suggesting hepatic overload.

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