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Oil blends with sesame oil in fish diets: oxidative stress status and fatty acid profiles of lambari

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ABSTRACT - The objective of this research was to evaluate the growth performance, oxidative stress, and fatty acid profiles of lambari (*Astyanax altiparanae*) fed diets containing different lipid sources: soybean oil, linseed oil, and freshwater fish residue oil combined or otherwise with sesame oil (SEO). The fish (mean weight 0.95±0.46 g; mean length 4.21±2.77 cm) were distributed into 24 cages (cage capacity: 0.70 m^3; fish density: 276 individuals m^{-3}) in six treatments and four replicates. After 80 days of feeding, they were weighed, and samples were collected for assay of catalase, glutathione reductase (GR), and lactate dehydrogenase (LDH) enzyme activities in muscle and analysis of the fatty acid profiles of polar and neutral fractions of whole eviscerated fish. The addition of SEO reduced docosahexaenoic acid (C22:6n-3, DHA) levels but increased the percentage of highly unsaturated n-3 fatty acids and the DHA: eiocosapentaenoic acid ratio, while reduced GR and LDH enzyme activities in muscle. Thus, certain blends of oils added to fish diets can improve the lipid profile of lambari and protect consumers against reactive oxygen species.

Keywords: fish, lipid, nutrition, oxidation

Introduction

The inclusion of large quantities of vegetable oils in aquafeed causes undesirable changes, such as increased levels of 18-carbon polyunsaturated fatty acids, mainly C18:2n-6 (linoleic acid, LA), and reduces the occurrence and quantity of highly unsaturated fatty acids (HUFA), such as C20:5n-3 (eicosapentaenoic acid, EPA) and C22:6n-3 (docosahexaenoic acid, DHA) (Ng et al., 2013). Sesame oil is considered as stable to oxidation due to its content of phenolic compounds such as sesamol derivatives (Turchini et al., 2009). Likewise, the addition of sesamin, a sesame lignan associated with sunflower and linseed oils, to aquafeed for rainbow trout (*Oncorhynchus mykiss*) promotes formation of DHA by acting on peroxisome β-oxidation (Trattner et al., 2008a).

Increased HUFA content in tissues elevates susceptibility to free radical production and lipid peroxidation (Ng et al., 2013). Studies demonstrate that lambari, *Astyanax altiparanae* (Garutti and

Britski, 2000), probably has the capacity to desaturate and elongate LA and linolenic acid (LNA), contained in its diet, to HUFA, such as arachidonic acid (AA), EPA, and DHA (Gonçalves et al., 2012).

Lambari (*Astyanax altiparanae*) is a Brazilian native omnivore freshwater fish species that has basic characteristics such as fast growth rate, easy adaptation to the intensive production system, and a promising market for human consumption and live bait (Gonçalves et al., 2014). It may, therefore, be useful to obtain information about the nutrition, physiology, and feeding behavior of this native Brazilian species (Abimorad and Castellani, 2011; Gonçalves et al., 2014), which, due to its small size, can be studied as a model for other larger species.

In a recent study, lambari fed feed containing plant oils combined with sesame oil showed a higher n-3 desaturation index and a lower hepatic peroxidation index, quantified by thiobarbituric acid reactive substances (TBARS) levels (Natori et al., 2016). However, the effect of sesame oil on oxidative stress enzymes and its effect on the polar and neutral fraction profiles of fish, when given in feed, were not investigated.

The objective of this research was to evaluate the effect of diets including soy oil (SO), linseed oil (LO), or freshwater fish residue oil (FRO) with or without sesame oil (SEO) on the oxidative stress status and polar and neutral lipid fraction composition of lambari. The experimental diets contained higher levels of EPA and DHA than the diets tested by Natori et al. (2016) and were supplemented with biotin and pantothenic acid.

Material and Methods

The study was conducted according to case no. 2012.1.1435.74.8 of the institutional committee on animal use. The experimental design was completely randomized for the two factors tested: three oils, i.e., SO (Cargill Brazil Company, São Paulo, SP, Brazil), LO (Cisbra Ltda, Panambi, RS, Brazil), and FRO (donated by Nosso Recanto Trout Farm, Sapucaí Mirim, MG, Brazil), with or without SEO (Sum Sum Hong, São Paulo, SP, Brazil), totalizing six treatments and four replicates for each treatment.

The lambari (mean weight 0.95±0.46 g; mean length 4.21±2.77 cm) (from Instituto de Pesca, Pirassununga, Brazil) were distributed into 24 cages (capacity 0.70 m^3) at a density of 276 fish m^{-3} corresponding to a biomass of 272.20 g m^{-3}, installed in two ponds in Pirassununga, São Paulo, Brazil (latitude 21°59'46" S, longitude 47°25'33" W, elevation 627 m). The fish were fed isoproteic (30 g 100 g^{-1}) and isocaloric (4239 kcal 100 g^{-1}) diets, which formulation (Table 1) was based on studies carried out by Campelo et al. (2015) and Sussel et al. (2014), supplemented with 3.0 g 100 g^{-1} of the different kinds of oil (SO, LO, or FRO) with or without 1.50 100 g^{-1} SEO (estimated to contain 37.50 mg 100 g^{-1} of sesamin/episamin; Trattner et al., 2008a). In addition, a quantity corresponding to 2 mg of biotin and 50 mg of calcium pantothenate was added to the diets. The chemical composition (Table 1) and fatty acids profile (Table 2) of experimental diets were determined by AOAC (1990) and AOAC (2005; method 966.06), respectively.

During the feeding trial, water quality parameters in the cages were monitored on alternate days. Temperature, dissolved oxygen, and pH were measured with a multi-parameter water quality meter (U-10 Horiba, Kyoto, Japan). Total and soluble phosphorus, nitrite, and nitrate levels were determined with specific kits (Hanna Instruments, Woonsocket, RI, USA).

After a period of 80 days of feeding twice a day (at 9:00 and 16:00 h), the individuals in each cage were anesthetized with clove oil (50 mg L^{-1} water) (Pereira-da-Silva et al., 2009), slaughtered by cranial drilling, and weighed. The following performance variables were calculated: feed intake, weight gain, feed conversion ratio, protein efficiency ratio, and specific growth rate. Muscle samples as well as eviscerated fish were analyzed as detailed below.

Catalase, glutathione reductase, and lactate dehydrogenase enzyme activities were assayed in white muscle homogenate from seven fish per treatment. Muscle samples (each 0.50 g) were homogenized with 1.25 mL cold 10 mM sodium phosphate buffer (Sigma Aldrich, Spruce St. Louis,

MO, USA) at pH 7.4 using a Potter type homogenizer (Tecnal, Piracicaba, SP, Brazil). The samples were centrifuged for 10 min at 10,000 rpm at 4 °C, and the supernatants were transferred to micro-tubes inserted in ice. The protein content of each sample was determined by the Bradford (1976) method, and bovine serum albumin (Sigma Aldrich, Spruce St. Louis, MO, USA) was used to obtain the standard curve.

Catalase (CAT) activity was measured as hydrogen peroxide (H_2O_2) consumption for 3 min at 25 °C (Beers and Sizer, 1952) and read at 240 nm using a DU-800 spectrophotometer (Beckman Coulter, Brea, CA, USA). Results were expressed in µmol of H_2O_2 per minute per mg of protein. For this analysis, 20 µL of each homogenate was added to assay medium containing 980 µL potassium phosphate buffer 50 mM pH 7.0 (code P5379, Sigma Aldrich, Spruce St. Louis, MO, USA) and H_2O_2 10 mM (code H1009 Sigma Aldrich, Spruce St. Louis, MO, USA).

Table 1 - Ingredients, formulation, and composition of the experimental diets for lambari

Item	Diet					
	SO	LO	FRO	SO+SEO	LO+SEO	FRO+SEO
Ingredient (g kg^{-1})						
Corn meal	267.1	267.1	267.1	267.1	267.1	267.1
Viscera meal	120.0	120.0	120.0	120.0	120.0	120.0
Soybean meal	137.3	137.3	137.3	137.3	137.3	137.3
Wheat meal	220.0	220.0	220.0	220.0	220.0	220.0
Sugar cane yeast	5.0	5.0	5.0	5.0	5.0	5.0
Rice meal	80.0	80.0	80.0	80.0	80.0	80.0
Meat meal	70.0	70.0	70.0	70.0	70.0	70.0
Fishmeal	55.0	55.0	55.0	55.0	55.0	55.0
Dicalcium phosphate	4.1	4.1	4.1	4.1	4.1	4.1
Soybean oil	30.0	-	-	15.0	-	-
Linseed oil	-	30.0	-	-	15.0	-
Freshwater fish residue oil	-	-	30.0	-	-	15.0
Sesame oil	-	-	-	15.0	15.0	15.0
Chlorine chloride	1.0	1.0	1.0	1.0	1.0	1.0
L-lysine	1.1	1.1	1.1	1.1	1.1	1.1
DL-methionine	1.3	1.3	1.3	1.3	1.3	1.3
Antioxidant	0.1	0.1	0.1	0.1	0.1	0.1
Antifungal	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin and mineral supplement[1]	5.0	5.0	5.0	5.0	5.0	5.0
Common salt	2.0	2.0	2.0	2.0	2.0	2.0
Chemical composition (g 100 g^{-1})						
Dry matter	96.59	96.58	97.26	97.21	96.36	97.29
Crude protein	30.45	31.69	30.39	29.52	30.18	30.16
Ether extract	6.88	7.68	8.19	8.44	8.35	8.26
Ash	10.16	10.34	10.04	10.19	10.07	10.13
Crude energy (MJ kg^{-1})	17.75	17.79	17.64	17.73	17.77	17.78

SO - soybean oil; LO - linseed oil; FRO - fresh water fish residue oil; SEO - sesame oil.
[1] Vitamin and mineral supplement (0.50 g kg^{-1}): vitamin A, 12,000 UI; vitamin D3, 3,000 UI; vitamin E, 150 mg; vitamin K3, 15 mg; vitamin B2, 20 mg; vitamin B6, 17.50 mg; vitamin B12, 40 µg; vitamin C, 300 mg; nicotinic acid, 100 mg; calcium pantothenate, 100 mg; biotin, 2.00 mg; folic acid, 6 mg; copper sulphate, 17.50 mg; iron sulfate, 100 mg; manganese sulphate, 50 mg; zinc sulfate, 120 mg; calcium iodide, 0.80 mg; sodium sulfate, 0.50 mg; cobalt sulphate, 0.40 mg; inositol, 125 mg; choline chloride, 500 mg.

Glutathione reductase (GR) activity was evaluated as NADPH oxidation and consequent reduction of oxidized glutathione (GSSG) at 25 °C for 3 min by spectrophotometry (Beckman Coulter, Pasadena, CA, USA) reading at 340 nm (Carlberg and Mannervik, 1985). Results were expressed in nmol of oxidized NADPH per minute per mg protein. For this analysis, a 40-µL aliquot of homogenate was added to an assay medium containing 500 µL potassium phosphate 100 mM pH 7.0 (code P5379, Sigma Aldrich, Spruce St. Louis, MO, USA) containing 1 mM EDTA (code E1644, Sigma Aldrich, Spruce St. Louis, MO, USA), 5 µL NADPH 0.10 mM (code N1630, Sigma Aldrich, Spruce St. Louis, MO, USA), and 50 µL GSSG 1.0 mM (code G6654, Sigma Aldrich, Spruce St. Louis, MO, USA).

Lactate dehydrogenase (LDH) activity was evaluated as oxidation of NADH at 25 °C for 3 min using a DU-800 Beckman Coulter spectrophotometer read at 340 nm (Bernt and Bergmeyer, 1974). Results were expressed in µmol of oxidized NADH per minute per mg protein. For this analysis, 10 µL diluted homogenate (1:50, v:v) were added to 990 µL assay medium containing 123.75 µL sodium phosphate buffer 20 mM pH 7.4 (code S8282, Sigma Aldrich, Spruce St. Louis, MO, USA), 12.37 µL pyruvic acid

Table 2 - Fatty acid profiles of the experimental diets for lambari (values are expressed in g 100 g^{-1} of total fatty acids)

Fatty acid	Diet					
	SO	LO	FRO	SO+SEO	LO+SEO	FRO+SEO
C12:0	0.04	0.03	0.07	0.00	0.00	0.10
C14:0	1.06	0.88	1.39	0.98	1.20	1.54
C15:0	0.17	0.18	0.20	0.19	0.28	0.25
C16:0	15.88	13.89	19.56	15.74	14.29	17.33
C16:1	2.24	1.92	5.20	2.07	2.56	4.37
C17:0	0.25	0.22	0.28	0.25	0.18	0.20
C18:0	4.62	5.63	5.88	5.23	4.41	4.67
C18:1n-9	24.77	23.13	31.15	28.95	24.32	29.86
C18:2n-6 (LA)	43.99	25.55	28.72	41.18	32.86	35.74
C18:3n-6	0.07	0.05	0.35	0.00	0.00	0.25
C18:3n-3 (LNA)	3.97	25.57	1.69	2.72	17.33	1.89
C20:0	0.19	0.17	0.16	0.23	0.12	0.10
C20:2n-6	0.06	0.06	0.55	0.05	0.00	0.26
C20:3n-6	0.07	0.05	0.52	0.08	0.00	0.26
C20:4n-6 (AA)	0.45	0.42	0.94	0.39	0.47	0.75
C20:3n-3	0.00	0.03	0.04	0.00	0.00	0.00
C20:5n-3 (EPA)	0.43	0.42	0.47	0.40	0.47	0.48
C22:0	0.16	0.11	0.06	0.13	0.00	0.05
C22:1n-9	0.00	0.02	0.10	0.00	0.00	0.00
C22:2n-6	0.00	0.00	0.05	0.00	0.00	0.00
C24:0	0.09	0.06	0.06	0.07	0.00	0.00
C22:6n-3 (DHA)	1.06	1.03	1.39	1.01	1.03	1.19
ΣSFA	22.46	21.17	27.66	22.82	20.48	24.24
ΣMUFA	27.01	25.07	36.45	31.02	26.88	34.23
ΣPUFA	50.04	53.12	34.12	45.78	52.16	40.56
ΣPUFAn-6	44.64	26.13	31.13	41.7	33.33	37.26
ΣPUFAn-3	5.46	27.05	3.59	4.13	18.83	3.56
ΣPUFAn-6:ΣPUFAn-3	8.18	0.97	8.67	10.1	1.77	10.47

SO - soybean oil; LO - linseed oil; FRO - fresh water fish residue oil; SEO - sesame oil; LA - linoleic acid; LNA - linolenic acid; AA - arachidonic acid; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.

1.0 mM (code P2256, Sigma Aldrich, Spruce St. Louis, MO, USA), 12.37 µL NADH 0.10 mM (code N8129, Sigma Aldrich, Spruce St. Louis, MO, USA), and 841.50 µL ultrapure water.

Fatty acid (FA) profile was determined in pools of 10 individuals per replicate, totalizing 40 animals (i.e., four pools) per treatment. For each pool, the fish were eviscerated, freeze-dried, minced, and stored in a box. Lipid extraction was done according to the method of Folch et al. (1957). The samples were homogenized in chloroform and methanol solution (2:1 v/v, with 0.01‰ BHT) and then filtered. The lipid extract was washed with 0.88% KCl solution, and the solvents removed by Rotavapor. The purified lipid extract was dissolved in 5 mL chloroform and stored in sealed amber glass bottles. Nitrogen was added to the bottles before sealing to prevent oxidation, which were stored in a freezer at −20 °C until analysis.

Neutral and polar lipid fractions were separated according to Juaneda and Rocquelin (1985) using a Sep-Pak® silica cartridge (Waters Company, Milford, MA, USA): 30 mg lipid dissolved in 500 µL chloroform (Sigma Aldrich, St. Louis, MO, USA) were applied to each cartridge. The cartridge was placed in a 50 mL syringe, and the neutral fraction was extracted by injecting 20 mL chloroform and 5 mL chloroform-methanol solution (49:1 v/v). The polar fraction (phospholipids) was extracted by injecting 30 mL methanol.

Fatty acid profiles of neutral and polar fractions were obtained in two steps: saponification with KOH 0.5 N in methanol and esterification with boron trifluoride-methanol solution (BF_3 14%; code B1252, Sigma Aldrich, St. Louis, MO, USA), according to Morrison and Smith (1964). The fatty acid methyl esters (FAME) obtained were analyzed by gas chromatography (model 430-GC with CP-8400 auto sampler; Varian Inc., Mitchell Drive Walnut Creek, CA, USA) with flame ionization detection (GC-FID) on an Omegawax® Capillary GC Column (30 m × 0.32 mm × 0.25 µm film thickness; code 24152 Supelco, Sigma Aldrich, Spruce St. Louis, MO, USA), using helium as carrier gas. The gas chromatograph temperature cycle was programmed as follows: 90 °C for 1 min; heat to 180 °C at 20 °C min^{-1}, and then to 220 °C at 3 °C min^{-1}. The duration of gas chromatograph analysis was 40 min for each sample.

The chromatograms were recorded with integrator software (Galaxie Chromatography Data System 1.9.302.952; Varian Inc., Mitchell Drive Walnut Creek, CA, USA) and the FA were identified by comparing the retention time of FAME with the standard C4-C24 FAME mix (code 18919 Supelco, Sigma Aldrich, Spruce St. Louis, MO, USA). Fatty acids were quantified through three levels of calibration curve, built using the same C4-C24 FAME mix with tricosanoic acid (C23:0; code T6543, Sigma Aldrich, Spruce St. Louis, MO, USA) as internal standard.

The data was analysed by the GLM procedure of SAS software (Statistical Analysis System, version 9.2), considering the two factors, i.e., kind of oil (three levels: SO, LO, FRO) and presence/absence of sesame oil (two levels: with or without), and their interaction in the statistical model. Significant differences between treatments were detected by F test.

Results

During the trial, temperature, dissolved oxygen, pH, total and soluble phosphorus, nitrites, and nitrates were in the following ranges: 26.53-28.55 °C, 6.39-6.90 mg L^{-1}, 5.58-6.35, 0.01-0.10 mg L^{-1}, 0.00-0.09 mg L^{-1}, 1.00-4.00 mg L^{-1}, and 0.00-1.50 mg L^{-1}, respectively.

As expected, the addition of SEO to SO, LO, or FRO did not cause any reduction in growth performance variables ($P>0.05$) (Table 3), but it determined a reduction in GR and LDH activities in muscle ($P<0.05$). The highest GR activity was found in fish fed the FRO diet. Catalase activity was similar in all groups, proving unaffected by the different lipid sources and by addition of SEO (Table 4).

The fatty acid profiles showed that SO, LO, and FRO plus SEO modified neutral and polar lipid profiles ($P<0.05$) (Tables 5 and 6).

A significant interaction between SEO and the kind of oil tested was found for the following fatty acids of neutral and polar lipids: linoleic acid (C18:2n-6), linolenic acid (C18:3n-3), arachidonic acid

(C20:4n-3), and eiocosapentaenoic acid (C20:5n-3). The higher polyunsaturated fatty acids (PUFA) and PUFAn-3 contents were detected in fish fed the LO diet.

The HUFAn-3:LNA ratio showed higher values in fish fed SEO, demonstrating their probable capacity to convert LNA into HUFAn-3. The DHA:EPA ratio was also lower in fish fed the LO diet than in those fed the LO+SEO diet (Tables 5 and 6). As expected, the polar fraction showed higher PUFA (AA, EPA, and DHA) than monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) contents, and lower PUFAn-6:PUFAn-3 ratios and lower percentages of LA and LNA than the neutral fraction.

Table 3 - Mean values of growth performance variables of lambari

Item	SEO	Oil source (OS)			Mean	P-value			RSD
		SO	LO	FRO		OS	SEO	OS×SEO	
	Without	7.94	7.81	7.48	7.74				
FI (g fish^{-1})	With	8.22	7.16	7.66	7.68	0.19	0.81	0.33	0.68
	Mean	8.08	7.48	7.57					
	Without	6.06	6.01	5.68	6.04				
WG (g fish^{-1})	With	6.10	5.39	5.50	5.81	0.32	0.36	0.40	0.61
	Mean	6.20	5.78	5.79					
	Without	1.31	1.30	1.32	1.28				
FCR	With	1.36	1.33	1.42	1.33	0.95	0.37	0.85	0.14
	Mean	1.31	1.30	1.32					
	Without	2.53	2.43	2.50	2.54				
PER	With	2.51	2.50	2.38	2.52	0.86	0.91	0.73	0.26
	Mean	2.57	2.50	2.53					
	Without	2.43	2.43	2.37	2.43				
SGR (% day^{-1})	With	2.44	2.31	2.32	2.38	0.32	0.30	0.39	0.11
	Mean	2.46	2.38	2.38					

SO - soybean oil; LO - linseed oil; FRO - fresh water fish residue oil; SEO - sesame oil; FI - feed intake; WG - weight gain; FC - feed conversion ratio; PER - protein efficiency ratio; SGR - specific growth rate; RSD - residual standard deviation.

Table 4 - Mean values of catalase activity (CAT) (µmol min^{-1} mg^{-1} protein), glutathione reductase activity (GR) (nmol min^{-1} mg^{-1} protein), and lactate dehydrogenase (LDH) (µmol min^{-1} mg^{-1} protein)

Item	SEO	Oil Source (OS)			Mean	P-value			RSD
		SO	LO	FRO		OS	SEO	OS×SEO	
	Without	0.79	0.73	0.71	0.74				
CAT	With	0.73	0.44	0.75	0.64	0.60	0.50	0.68	0.52
	Mean	0.76	0.58	0.73					
	Without	1.48	1.49	2.61	1.86a				
GR	With	1.41	1.32	1.60	1.44b	0.005	0.031	0.09	0.63
	Mean	1.44B	1.40B	2.10A					
	Without	4.98	4.92	4.82	4.91a				
LDH	With	4.83	3.96	3.79	4.19b	0.36	0.046	0.53	1.21
	Mean	4.91	4.44	4.31					

SO - soybean oil; LO - linseed oil; FRO - fresh water fish residue oil; SEO - sesame oil; RSD - residual standard deviation.
Within criterion, mean values with different lowercase and uppercase letters in columns and rows, respectively, are significantly different at P<0.05.

Table 5 - Main fatty acids, fatty acid groups, and relationships in the neutral lipid fraction of lambari (values are expressed in g 100 g^{-1} of total fatty acids)

Item	SEO	Oil Source (OS)			Mean	P-value			RSD
		SO	LO	FRO		OS	SEO	OS×SEO	
	Without	0.98a	0.86b	0.90a	0.91				
C12:0	With	0.90b	0.87a	0.91a	0.85	0.0002	0.22	0.02	0.03
	Mean	0.94	0.86	0.90					
	Without	1.77	1.60	1.75	1.71				
C14:0	With	1.70	1.65	1.77	1.70	<0.0001	0.92	0.05	0.044
	Mean	1.73A	1.63B	1.76A					
	Without	18.56	17.57	19.13	18.42				
C16:0	With	18.62	18.26	18.98	18.62	0.01	0.48	0.45	0.68
	Mean	18.59AB	17.91B	19.05A					
	Without	7.85	8.10	8.13	8.03				
C18:0	With	814	8.17	7.92	8.07	0.39	0.58	0.08	0.20
	Mean	7.99	8.13	8.03					
	Without	36.67	36.05	38.06	36.92				
C18:1n-9	With	37.68	36.93	37.59	37.40	0.22	0.44	0.56	1.49
	Mean	37.18	36.49	37.83					
	Without	14.11a	10.38a	10.79a	14.10				
C18:2n-6	With	12.26b	11.58a	11.48a	12.26	<0.001	0.98	0.004	0.83
	Mean	13.18	10.98	11.13					
	Without	1.51a	6.36a	1.09a	2.99				
C18:3n-3	With	1.15b	3.48b	0.98a	1.87	<0.001	<0.001	<0.001	0.13
	Mean	1.33	4.92	1.04					
	Without	0.80a	0.56b	0.84a	0.74				
C20:4n-6	With	0.78a	0.66a	0.85a	0.76	<0.0001	0.09	0.01	0.04
	Mean	0.79	0.61	0.84					
	Without	0.42a	0.57a	0.36a	0.42				
C20:5n-3	With	0.37b	0.47b	0.37a	0.37	<0.0001	<0.0001	0.0004	0.02
	Mean	0.39	0.52	0.36					
	Without	1.48	1.63	1.36	1.49a				
C22:6n-3	With	1.33	1.49	1.31	1.37b	<0.0001	<0.0001	0.17	0.05
	Mean	1.40B	1.56A	1.33C					
	Without	30.27	30.86	32.73	31.28				
ΣSFA	With	32.18	31.70	32.43	31.10	0.15	0.20	0.36	1.51
	Mean	31.22	31.28	32.58					
	Without	44.39	43.10	46.45	44.64				
ΣMUFA	With	45.34	44.17	45.84	45.11	0.01	0.46	0.49	1.54
	Mean	44.86AB	43.63B	46.14A					
	Without	25.35a	26.04a	20.82a	24.07				
ΣPUFA	With	22.48b	24.12b	21.73a	22.77	<0.0001	0.007	0.005	1.03
	Mean	23.91	25.08	21.27					
	Without	17.74a	13.11b	14.40a	15.08				
ΣPUFAn-6	With	15.70b	14.60a	15.06a	15.11	<0.0001	0.92	0.001	0.82
	Mean	16.72	13.85	14.73					

Continues...

Table 5 (Continued)

	Without	4.81a	10.32a	4.04a	6.39				
ΣPUFAn-3	With	4.10b	6.93b	3.94a	4.99	<0.0001	<0.0001	<0.0001	0.16
	Mean	4.45	8.62	3.99					
	Without	3.69b	1.27b	3.56a	2.84				
PUFAn-6: PUFAn-3	With	3.83a	2.10a	3.83a	3.25	<0.0001	<0.0001	0.0008	0.16
	Mean	3.76	1.68	3.70					
	Without	0.79a	0.84a	0.64a	0.75				
PUFA:SFA	With	0.70a	0.76b	0.67a	0.71	<0.0001	0.016	0.017	0.04
	Mean	0.74	0.80	0.65					
	Without	3.51a	2.87b	3.74a	3.37				
DHA:EPA	With	3.64a	3.15a	3.57b	3.45	<0.0001	0.16	0.007	0.13
	Mean	3.57	3.01	3.66					
	Without	2.19	0.62	3.15	1.84b				
HUFAn-3: LNA	With	2.57	0.99	3.57	2.18a	<0.0001	<0.0001	0.83	0.97
	Mean	2.37B	0.81C	2.85A					
	Without	0.26	0.26	0.34	0.28				
HUFAn-6:LA	With	0.28	0.26	0.31	0.28	0.0001	1.00	0.13	0.02
	Mean	0.27B	0.26B	0.32A					

SO - soybean oil; LO - linseed oil; FRO - fresh water fish residue oil; SEO - sesame oil; LA - linoleic acid; LNA - linolenic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; HUFA - high-unsaturated fatty acids; DHA - docosahexaenoic acid; EPA - eicosapentaenoic acid; RSD - residual standard deviation.
Within criterion, mean values with different lowercase and uppercase letters within columns and rows, respectively, are significantly different at P<0.05.

Discussion

The lipid sources used in this trial proved suitable for addition to fish feed in different blends without affecting fish growth and production. Soy oil and linseed oil were chosen due to their different fatty acid compositions, namely predominance of LA and LNA, respectively. The partial substitution of fish oil with such plant oils in diets for marine and freshwater fish species has occurred in recent years (Nasopoulou and Zabetakis, 2012), due the high price of fish oil and the poor sustainability of conventional aquafeeds based on this ingredient.

Fish residue oil was included to evaluate the suitability of freshwater fish production and processing wastes as an ingredient for aquafeeds. The lipid profiles of oils extracted from animal wastes have a high oleic acid (C18:1n-9) content (Bureau and Meeker, 2010; Gonçalves et al., 2012).

The fatty acid profiles in the polar and neutral fractions were determined to verify the effects of dietary SEO additions, regarding the LA, LNA, and HUFA of the families n-3 and n-6 percentages. According to Alhazzaa et al. (2018), fish as other vertebrates must be fed diets with preformed n-3 HUFA (EPA, DHA) and n-6 HUFA (AA) or the precursors (LA and LNA, respectively). However, the fatty acids quantities in diets depend on the capacity of each species to desaturate and elongate the LA and LNA, determined by the relative activity, availability, and affinity levels of their enzymes.

Besides having beneficial proprieties, SEO can also be used as a lipid source in fish diets. The combination of sunflower oil, linseed oil, fish residue oil, and SEO in diets of rainbow trout did not reduce weight gain or specific growth rate and did not increase the feed conversion rate (Köse and Yildiz, 2013). However, early juveniles of *Lates calcarifer*, a euryhaline catadromous species, responded to addition of sesamin to the diet with growth reduction (Alhazzaa et al., 2012).

Lambari, also known as yellowtail tetra, can adjust its gastrointestinal tract activity to utilize different ingredients (Sussel et al., 2014), such as the different oils used in this trial. Addition of oil extracted

Table 6 - Main fatty acids, fatty acid groups, and relationships in the polar lipid fraction of lambari (values are expressed in g 100 g^{-1} of total fatty acids)

Item	SEO	Oil Source (OS)			Mean	P-value			RSD
		SO	LO	FRO		OS	SEO	OS×SEO	
	Without	1.31	1.30	1.32	1.31a				
C12:0	With	1.23	1.21	1.12	1.18b	0.74	0.03	0.62	0.12
	Mean	1.27	1.25	1.22					
	Without	1.63	1.66	1.70	1.66				
C14:0	With	1.63	1.52	1.58	1.58	0.60	0.06	0.40	0.10
	Mean	1.63	1.59	1.64					
	Without	13.68	14.18	14.54	14.14b				
C16:0	With	14.53	14.81	15.32	14.88a	0.04	0.006	0.91	0.59
	Mean	14.10B	14.50AB	14.93A					
	Without	8.50	8.57	8.53	8.53				
C18:0	With	8.58	8.74	8.80	8.70	0.68	0.19	0.84	0.31
	Mean	8.54	8.65	8.66					
	Without	17.01	17.81	17.49	17.44				
C18:1n-9	With	18.59	18.65	19.54	18.93	0.61	0.02	0.70	1.42
	Mean	17.80	18.23	18.52					
	Without	7.71a	6.23a	6.06b	6.66				
C18:2n-6	With	7.31a	7.06a	7.12a	7.16	0.008	0.046	0.044	0.57
	Mean	7.51	6.64	6.59					
	Without	0.88a	2.48a	0.75a	1.37				
C18:3n-3	With	0.75b	1.54b	0.71a	1.01	<0.0001	<0.0001	<0.0001	0.12
	Mean	0.84	2.01	0.73					
	Without	7.26a	4.11b	7.70a	6.36				
C20:4n-6	With	7.40a	5.77a	7.69a	6.96	<0.0001	0.005	0.003	0.46
	Mean	7.33	4.94	7.70					
	Without	1.49a	2.87a	1.23a	1.87				
C20:5n-3	With	1.26a	2.25b	1.20a	1.57	<0.0001	<0.0001	0.0005	0.12
	Mean	1.38	2.56	1.22					
	Without	14.69	17.19	13.73	15.20a				
C22:6n-3	With	13.29	15.76	12.20	13.73b	<0.0001	0.002	0.99	0.75
	Mean	13.99B	16.48A	12.96B					
	Without	28.86	29.55	30.04	29.48				
ΣSFA	With	29.68	29.67	29.97	29.77	0.046	0.21	0.25	0.54
	Mean	29.27B	29.61AB	30.00A					
	Without	23.87	24.63	24.75	24.41				
ΣMUFA	With	25.55	24.72	26.32	25.53	0.38	0.06	0.44	1.37
	Mean	24.71	24.67	25.54					
	Without	47.27	45.82	45.20	46.09a				
ΣPUFA	With	44.77	45.61	43.71	44.69b	0.15	0.049	0.38	1.62
	Mean	46.02	45.71	44.45					
	Without	23.50a	15.88b	22.78a	20.71				
ΣPUFAn-6	With	23.22a	19.57a	23.75a	22.18	<0.0001	<0.0001	<0.0001	0.71
	Mean	23.36	17.73	23.24					

Continues...

Table 6 (Continued)

	Without	19.50	25.73	18.04	21.10a				
ΣPUFAn-3	With	17.50	22.15	16.13	18.59b	<0.0001	<0.0001	0.29	1.14
	Mean	18.50B	23.94A	17.08C					
	Without	1.21	0.62	1.26	1.02b				
PUFAn-6: PUFAn-3	With	1.33	0.88	1.48	1.24a	<0.0001	<0.0001	0.22	0.08
	Mean	1.26B	0.75C	1.37A					
	Without	1.64	1.55	1.50	1.54				
PUFA:SFA	With	1.50	1.53	1.46	1.50	0.08	0.05	0.28	0.07
	Mean	1.57	1.54	1.48					
	Without	9.84a	5.99b	11.17a	8.99				
DHA:EPA	With	10.64a	7.01a	10.19a	9.28	<0.0001	0.30	0.01	0.66
	Mean	10.24	6.50	10.68					
	Without	21.17a	9.48a	23.00a	17.87				
HUFAn-3: LNA	With	21.23a	13.49b	21.59a	18.77	<0.0001	0.29	0.04	2.01
	Mean	21.20	11.49	22.30					
	Without	2.06	1.55	2.79	2.13				
HUFAn-6:LA	With	2.20	1.78	2.34	2.11	<0.0001	0.81	0.06	0.29
	Mean	2.13B	1.67C	2.56A					

SO - soybean oil; LO - linseed oil; FRO - fresh water fish residue oil; SEO - sesame oil; LA - linoleic acid; LNA - linolenic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; HUFA - high-unsaturated fatty acids; DHA - docosahexaenoic acid; EPA - eicosapentaenoic acid; RSD - residual standard deviation.
Within criterion, mean values with different lowercase and uppercase letters within columns and rows, respectively, are significantly different at $P<0.05$.

from salmon and tilapia residues to feed did not affect the growth and development of lambari (Gonçalves et al., 2012). This species can also assimilate synthetic compounds such as conjugated linoleic acid without growth reduction or decreased specific growth rate and with increased feed conversion (Campelo et al., 2015).

The different types of oil in lambari feed proved to be suitable alternative lipid sources that modulate fish physiology and the chemical composition of their flesh. In a previous study (Natori et al., 2016), lambari fed a diet including LO showed higher TBARS formation than those fed diets containing LO combined with SEO. The ability of SEO to reduce oxidation processes was also demonstrated in diabetic rats. When fed diets containing SEO, free radical formation decreased; moreover, the sesamolin contained in SEO reduced hepatic TBARS values in normal rats (Kang et al., 1998; Ramesh et al., 2005).

In the marine species Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua*), the inclusion of highly unsaturated and oxidized oil in feed increased hepatic TBARS due to intestinal absorption of malondialdehyde or due to hydroperoxides formed by peroxidation of fatty acids present in tissues (Hamre et al., 2001; Kjær et al., 2008). In our trial, lambari fed FRO diets showed higher GR activities than those fed SO and LO diets, suggesting that the oxidation level of FRO affected the values of this variable.

Reduced GR activity in muscle of animals fed SEO diet can be due to the probable presence of bioactive compounds such as sesamin/episamin and tocopherols (Fukuda et al., 1986; Chung et al., 2004). Sesamin is metabolized in the liver and becomes effective at reducing free radicals such as superoxides. However, at high concentrations, this compound may cause oxidative damage (Jeng and Hou, 2005).

The lower enzyme activity of lactate dehydrogenase (LDH) after addition of SEO to the diet could also be related to the presence of sesamin lignan. In neurons, sesamin inhibits free radical formation and downregulates the release and activity of LDH, activated by hypoxia, preserving CAT and superoxide dismutase activities (Jeng and Hou, 2005). The lignan compound in SEO can modulate the formation

and quantities of DHA, as demonstrated by studies with rainbow trout muscle, Atlantic salmon hepatocytes, and *Lates calcarifer* fillets (Trattner et al., 2008a,b; Alhazzaa et al., 2012). In an *in vitro* study, the expression of CTP1, involved in β-oxidation, was higher in hepatocytes of Atlantic salmon fed sesamin in the diet (Trattner et al., 2008b).

The neutral fraction of lipids consists mainly of triglycerides stocked in adipose tissue, and their composition is influenced by species, season, and diet. The polar fraction, consisting mainly of phospholipids and glycolipids, composes the membrane structures and is highly susceptible to the lipid peroxidation due to its high PUFA content (Bao and Ohshima, 2014).

Differences in neutral fatty acid composition between treatments may influence fish growth maintenance and other physiological events such as reproduction or even the immune system (Turchini et al., 2010). Phospholipids are important components of the lipid profile, since they establish membrane fluidity and have a role in controlling cell functions (Clandinin et al., 1991). Differences in LA, LNA, AA, EPA, and DHA percentages caused by different oils in fish diets may alter some fish functions such as eicosanoid synthesis, the epidermal barrier, and free radical formation (Spector, 1999).

Similar effects of SEO and its combinations were also observed in the polar and neutral fractions. In rainbow trout, the combination of SEO, LO, and sunflower oil in fish feed promoted formation of C20:3n-3, demonstrating desaturation and elongation capacity through activation of Δ5 and Δ6 desaturase (Köse and Yildiz, 2013). In the present study, the addition of SEO to feed may have interfered with the quantities of EPA and DHA, due to the probable higher desaturation rate of LNA to HUFAn-3 and the higher DHA:EPA ratio in fish fed the LO+SEO diet than in fish fed the LO diet. Oxidation of fatty acids produces hydroperoxides by activation of PPARα and other secondary compounds (Takahashi et al., 2002). In our study, fish fed the LO diet showed a higher proportion of PUFA and a low PUFAn-6:PUFAn-3 ratio. This result may be related to the capacity of LO to produce LNA and, consequently, to promote an increase in EPA and DHA levels (Tocher, 2003).

The PUFAn-6:PUFAn-3 ratio is higher in freshwater than in salt water species, due to the higher proportion of PUFAn-6, mainly in the form of linoleic acid. According to Valfré et al. (2003), the PUFAn-6:PUFAn-3 ratio normally varies between 1 and 4. The higher MUFA and lower PUFA contents found in lambari fed the FRO and FRO+SEO diets are related to the higher level of oleic acid in waste generated by freshwater fish production and processing, from which FRO was obtained.

The effects of the alternative oil sources tested in this trial on the fatty acid profile of lambari depend on the physiological characteristics of this tropical fish species. As other tropical freshwater species, lambari probably can desaturate and elongate LA into AA and LNA into EPA and DHA, thus modifying dietary fatty acids by a metabolic process (Gonçalves et al., 2012).

Conclusions

The addition of sesame oil to the alternative lipid sources tested in this trial (i.e., soybean oil, linseed oil, and freshwater fish residue oil) may improve the fatty acid profile of lambari and may increase the desaturation rate of linolenic acid into HUFAn-3 levels of the polar and neutral fractions. The addition also protects these fatty acids from oxidation by alleviating the negative effects of oxygen free radicals in muscle, without impairing fish performance. Linseed oil promotes better fatty acid profile but may induce higher susceptibility to free radicals.

Furthermore, sesame oil added to the lambari diet improves fish quality by the probable increased production of highly unsaturated fatty acids and by reducing the activity of oxidative enzymes such as glutathione reductase and lactate dehydrogenase, without damaging the growth of fish.

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