Sesame oil in diets for lambari: Effects on growth parameters, corporal chemical composition and physiological alterations

Óleo de gergelim em dietas para lambari: Efeitos sobre o desempenho zootécnico, composição química corporal e alterações fisiológicas

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ABSTRACT

The inclusion of sesame oil associated with soybean, linseed and freshwater fish residue oil in the diets fed to Lambaris Astyanax altiparanae was evaluated by the growth performance parameters, body composition and possible physiological changes (GARUTTI & BRISTSKI, 2000). The experiment was a completely randomized design in two factorial parameters tested: three oil types (soy oil (SO), linseed oil (LO) and freshwater fish residue oil (FRO)), combined or not with sesame oil (SEO), totaling six treatments and four replications 24 cages, capacity of 0.70m³ (density of 251 fish m⁻³). The fish (mean weight 2.35g±0.62g and mean length 5.25cm±0.68cm) were fed with the experimental diets twice a day. After 75 days, the following parameters were determined: body chemical composition and fatty acid profile, glycemia, liver (LG) and muscle glycogen (MG) levels, and lipid peroxidation (TBARS). Fish fed with diets containing SEO had higher desaturation index values of LNA while those fed with SEO combined with LO displayed reduced hepatic lipid oxidation. Inclusion of SEO improved the fatty acid profile and stability, without causing problems related to fish performance and health.

Keywords: Astyanax altiparanae, fatty acids, nutrition, sesamin.

INTRODUCTION

The interest for vegetal oils as an alternative lipid source has increased (NAYLOR et al., 2000) because the inclusion of fishmeal and fish oil in the diet fed to fish led to increasing ration prices. However, it has been reported that in some marine fish species, the substitution of these ingredients can interfere with growth parameters, cause problems related to fish metabolism and consequently, the oxidative homeostasis, promoting the appearance of reactive oxygen species (ROS) (OLSVIK et al., 2011).

In tropical fish species such as Nile tilapia Oreochromis niloticus, the linolenic acid (LNA)
included in the diets is converted to high unsaturated fatty acid (HUFA) such as the docosahexanoic acid (DHA). The LNA addition decreased the linoleic acid (LA) desaturation to HUFA n-6 due to the high affinity of the enzymes involved in desaturation and elongation processes with the n-3 fatty acids (CHEN et al., 2013). Likewise, the lambari *Astyanax altiparanae* (Garutti & Bristski, 2000) is also able to convert LNA into eicosapentaenoic acid (EPA) and DHA, and LA into arachidonic acid (AA) (GONÇALVES et al., 2012; CAMPELO et al., 2014).

The therapeutic properties of sesam oil (SEO) have been verified in experiments with rats. The SEO-containing diets reduced glycemia, blood cholesterol and triglyceride levels and prevented pathological problems like formation of hepatic steatosis, and renal sepsis caused by lipopolysaccharide (LPS) (HSU et al., 2005; GUIMARÃES & MACEDO, 2013; PERIASAMY et al., 2014). Moreover, the lignans contained in SEO such as sesamin, episamin and sesamolin, may promote the formation of fatty acids as the DHA, from the desaturation and elongation of LNA (TRATTNER et al., 2008a; KÖSE & YILDIZ, 2013).

Therefore, this study evaluated how the inclusion of sesame oil associated with soy oil (SO), linseed oil (LO) or FRO (freshwater fish residue oil) in diets fed to lambari *Astyanax altiparanae* (Garutti & Bristski, 2000) affected the growth performance parameters, body chemical composition and determined possible physiological alterations.

**MATERIALS AND METHODS**

Table 1 shows the formulation (COTAN et al., 2006) and composition of the extruded

| Ingredients (%) | SO* | LO* | FRO* | SO/SEO* | LO/SEO* | FRO/SEO* |
|-----------------|-----|-----|------|---------|---------|---------|
| Corn meal       | 26.71 | 26.71 | 26.71 | 26.71 | 26.71 |
| Viscera meal    | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 |
| Soy meal        | 13.73 | 13.73 | 13.73 | 13.73 | 13.73 |
| Wheat meal      | 22.00 | 22.00 | 22.00 | 22.00 | 22.00 |
| Sugar cane yeast| 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Rice meal       | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Meat meal       | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Fish meal       | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 |
| Dicalcium phosphate | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 |
| Soy oil (SO)    | 3.00 | - | - | 1.50 | - |
| Linseed oil (LO) | - | 3.00 | - | - | 1.50 |
| Freshwater fish residue oil (FRO) | - | - | 3.00 | - | - |
| Sesame oil (SEO) | - | - | - | 1.50 | 1.50 |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| L-Lysine        | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 |
| DL-Methionine   | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 |
| Antioxidant     | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Antifungal      | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin and mineral supplement | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Common salt     | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |

**Chemical Composition (%)**

| Dry matter | 94.19 | 94.35 | 95.70 | 95.54 | 94.78 | 94.06 |
| Crude protein | 29.39 | 29.65 | 29.09 | 29.92 | 29.29 | 29.28 |
| Crude energy (kcal kg⁻¹) | 4375 | 4365 | 4395 | 4365 | 4385 | 4414 |
| Ether extract | 9.17 | 9.24 | 9.13 | 9.20 | 9.29 | 9.24 |
| Mineral matter | 10.18 | 10.35 | 10.15 | 10.02 | 10.00 | 10.18 |

*1 Treatments: Soy oil (SO 3%), linseed oil (LO 3%), freshwater fish residue oil (FRO 3%), soy oil and sesame oil (SO/SEO, 1.5%/1.5%), linseed oil and sesame oil (LO/SEO, 1.5%/1.5%), freshwater fish residue oil and sesame oil (FRO/SEO, 1.5%/1.5%). 1 Vitamin and mineral supplement (0.50g/100g): vitamin A, 12,000UI; vitamin D3, 3,000UI; Vitamin E, 150mg; Vitamin K3, 15mg; Vitamin B2, 20mg; Vitamin B6, 17.50mg; Vitamin B12, 40mcg; Vitamin C, 300mg; Nicotinic acid, 100mg; Calcium pantotenate, 50mg; Biotin, 1.00mg; Folic acid, 6mg; Sulphate Copper, 17.50mg; Iron sulphate, 100mg; Manganese sulphate, 50mg; Zinc sulphate, 120mg; Calcium iodide, 0.80mg; Sodium Sulfate, 0.50mg; Cobalt sulphate, 0.40mg; Inositol 125mg; Choline Chloride, 500mg; vehicle.
experimental diets (A.O.A.C., 1990), and lipid profiles (A.O.A.C., 2005) are shown in table 2. During the experimental period of 75 days, 4,224 fish (mean weight 0.95g±0.46g and mean length 4.21cm±2.77cm) were distributed in 24 cages (capacity 0.70m$^3$, density 251 fishes m$^{-3}$). Fish were fed ad libitum twice a day (at 9:00 and 16:00). Water quality parameters such as temperature, dissolved oxygen rate, and pH were measured using the Multi-Parameter Water Quality Monitoring Meter (Horiba U-10), on alternate days. Ammonia and nitrite levels were monitored using kits and meters from Hanna Instruments, United States. Fish were weighed on days 0, 35 and 75, anesthetized with clove oil (50mg L$^{-1}$ of water) (PEREIRA-DA-SILVA et al., 2009) and euthanized via cranial drilling.

The sampled fish were collected from those that fasted for 24 hours prior to sampling. The following variables were determined: diet consumption (DC), apparent feed conversion (AFC), weight gain (WG), specific growth rate (SGR) and protein efficiency rate (PER). The body chemical composition of whole fish was performed following the A.O.A.C. (1990) methodology to determine dry matter (DM), crude protein (CP), mineral matter (MM), ethereal extract (EE), and crude energy (CE).

A total of 25 fish were sampled per experimental unit, eviscerated and used to determine the fatty acid profile following the A.O.A.C. (2005) methodology. Lipid was extracted according to the BLIGH & DYER (1959) methodology.

Fish glycemia levels (n=10 per treatment) were measured by a portable glucose meter (Roche, Darmstadt, Germany).

### Table 2 - Lipid profile of experimental diets, expressed in g/100g of total fatty acids.

| Fatty acids | SO | LO | FRO | SO/SEO | LO/SEO | FRO/SEO |
|-------------|----|----|-----|--------|--------|---------|
| C12:0       | 0.00 | 0.06 | 0.01 | 0.00 | 0.00 | 0.00 |
| C14:0       | 0.67 | 0.50 | 1.29 | 0.56 | 0.52 | 0.97 |
| C14:1       | 0.05 | 0.04 | 0.13 | 0.07 | 0.06 | 0.27 |
| C15:0       | 0.00 | 0.09 | 0.14 | 0.11 | 0.13 | 0.14 |
| C16:0       | 13.97 | 10.89 | 19.78 | 13.68 | 11.62 | 16.07 |
| C16:1       | 1.42 | 1.19 | 5.70 | 1.15 | 1.06 | 3.69 |
| C17:0       | 0.18 | 0.27 | 0.21 | 0.14 | 0.14 | 0.16 |
| C18:0       | 3.51 | 4.81 | 5.65 | 4.77 | 4.7 | 5.21 |
| C18:1-n9 (cis+trans) | 24.40 | 21.30 | 33.41 | 29.90 | 26.91 | 33.50 |
| C18:2n-6    | 49.20 | 22.69 | 26.37 | 45.06 | 31.64 | 35.15 |
| C18:3n-6    | 0.00 | 0.03 | 0.51 | 0.00 | 0.00 | 0.28 |
| C18:3n-3    | 5.39 | 36.79 | 1.83 | 3.15 | 21.89 | 1.55 |
| C20:0       | 0.16 | 0.15 | 0.16 | 0.28 | 0.22 | 0.29 |
| C20:2n-6    | 0.00 | 0.05 | 0.72 | 0.00 | 0.00 | 0.35 |
| C20:3n-6    | 0.00 | 0.04 | 0.68 | 0.00 | 0.00 | 0.32 |
| C20:4n-6    | 0.26 | 0.23 | 0.98 | 0.22 | 0.21 | 0.59 |
| C20:5n-3    | 0.19 | 0.12 | 0.15 | 0.15 | 0.13 | 0.20 |
| C22:0       | 0.04 | 0.09 | 0.09 | 0.16 | 0.10 | 0.12 |
| C22:1n-9    | 0.00 | 0.01 | 0.11 | 0.00 | 0.00 | 0.00 |
| C22:2n-6    | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 |
| C24:0       | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| C22:6n-3    | 0.20 | 0.20 | 0.64 | 0.25 | 0.25 | 0.48 |
| ΣSFA$^1$    | 18.53 | 16.91 | 27.33 | 19.70 | 17.43 | 22.96 |
| ΣMUFA$^1$   | 25.87 | 22.54 | 39.35 | 31.12 | 28.03 | 37.46 |
| ΣPUFA$^1$   | 55.24 | 60.15 | 31.93 | 48.83 | 54.12 | 38.92 |
| PUFA4n-6    | 49.46 | 23.04 | 29.31 | 45.28 | 31.85 | 36.69 |
| PUFA4n-3    | 5.78 | 37.11 | 2.62 | 3.55 | 22.27 | 2.23 |
| PUFA4n-6n-3 | 8.56 | 0.62 | 11.19 | 12.75 | 1.43 | 16.45 |

*Treatments: Soy oil (SO 3%), linseed oil (LO 3%), freshwater fish residue oil (FRO 3%), soy oil and sesame oil (SO/SEO, 1.5%/1.5%), linseed oil and sesame oil (LO/SEO, 1.5%/1.5%), freshwater fish residue oil and sesame oil (FRO/SEO, 1.5%/1.5%). SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polysaturated fatty acids.
Brazil) in a 5-µL blood aliquot that was collected in the caudal vein region using heparinized insulin type syringes. The glycogen was quantified in 10mg of liver samples (LG) (n=14 per treatment) and 100mg of muscles (MG) (n=4 per treatment) following DUBOIS et al. (1956) modified by BIDINOTTO et al. (1997).

The thiobarbituric acid reactive substances (TBARS) were determined in hepatic tissues (n=6 per treatment) following the VYNCKE (1970) methodology adapted to microplate reader by modifying the proportions described by this author. The data were submitted to ANOVA (GLM procedure), using the SAS (2002) software. The F-test results showed significant differences among treatments. The LG and TBARS data were previously treated by the SAS/LAB command.

RESULTS AND DISCUSSION

Water quality parameters such as temperature, dissolved oxygen and pH were kept between 23.42 and 27.07ºC; 3.62 and 6.45mg L$^{-1}$; and 6.63 and 7.23, respectively. Ammonia and nitrite levels did not exceed 0.34 and 0.41mg L$^{-1}$, respectively. The growth parameters and body chemical composition (P<0.05) were not significantly different for fish fed with diets containing SO, LO and FRO, combined or not with SEO. However, the addition of SEO to the diets affected significantly glycemia and lipid peroxidation of hepatic tissue (TBARS) (Table 3). In addition, independent of the treatment, the glycemic levels above 49mg DL$^{-1}$ (PEREIRA-DA-SILVA et al., 2014) indicated that the fish underwent handling and capture stress during sampling (BARTON, 2002). Addition of SEO to the LO diet increased the blood glucose levels in fish. For the lambari, the lipid sources did not change the hepatic and muscular glycogen levels significantly. However, some fatty acids may have interfered with glycogen formation, such as the oleic acid, which modifies the activity of the enzymes involved in the glucose metabolism and hepatic reserves of rainbow trout (LIBRÁN-PÉREZ et al., 2013).

| Table 3 - Mean values of growth performance variables, body chemical composition and physiologic state. |
|-------------------------------------------------------------|---------------------------------------------------------------|
| Growth performance                                          | Treatments$^{*}$                                               |
| DC$^{2}$ (g)                                                | SO  | SO/SEO | LO  | LO/SEO | FRO  | FRO/SEO | P- value |
| 6.89                                                        | 7.02 | 6.71    | 6.98 | 7.46   | 7.40  | 0.51$^{ns}$ | 0.62     |
| WG$^{2}$ (g)                                                | 3.89 | 4.40    | 4.25 | 4.11   | 5.20  | 4.52     | 0.24$^{ns}$ | 0.74     |
| AFC$^{2}$                                                   | 1.61 | 1.54    | 1.57 | 1.61   | 1.46  | 1.53     | 0.40$^{ns}$ | 0.11     |
| PER$^{2}$                                                   | 1.93 | 2.00    | 2.13 | 1.99   | 2.40  | 2.09     | 0.39$^{ns}$ | 0.31     |
| SGR$^{2}$ (% / Day)                                         | 1.29 | 1.4     | 1.37 | 1.33   | 1.55  | 1.42     | 0.28$^{ns}$ | 0.15     |
| Chemical composition (%)                                    | SO  | SO/SEO | LO  | LO/SEO | FRO  | FRO/SEO | P- value |
| DM$^{2}$                                                   | 34.78 | 35.46  | 35.41 | 35.51 | 35.64 | 35.41 | 0.74$^{ns}$ | 0.82     |
| CP$^{2}$                                                    | 16.69 | 17.05  | 16.74 | 16.89 | 16.47 | 16.69 | 0.73$^{ns}$ | 0.53     |
| MM$^{2}$                                                    | 3.78 | 3.71    | 3.89 | 3.88   | 3.9   | 3.83     | 0.56$^{ns}$ | 0.17     |
| EE$^{2}$                                                    | 13.6 | 15.09   | 14.24 | 14.8  | 14.39 | 14.29 | 0.30$^{ns}$ | 0.70     |
| CE$^{2}$ (kcal 100g$^{-1}$)                                 | 226.3 | 232.05 | 229.47 | 230.75 | 231.58 | 228.14 | 0.87$^{ns}$ | 7.26     |
| Physiologic state                                          | SO  | SO/SEO | LO  | LO/SEO | FRO  | FRO/SEO | P- value |
| MG$^{2}$ (mg glicosyl g$^{-1}$)                             | 6.99 | 6.18    | 5.46 | 6.29   | 7.56  | 7.51     | 0.59$^{ns}$ | 1.77     |
| LG$^{2}$ (mg glicosyl g$^{-1}$)                             | 157.06 | 103.41 | 122.05 | 128.21 | 111.03 | 106.71 | 0.31$^{ns}$ | 61       |
| Interaction Effect Oil types$^{*}$ SEO                     |                                               |
| Physiologic state                                          | SO  | SO/SEO | LO  | LO/SEO | FRO  | FRO/SEO | P- value |
| Glycaemia (mg DL$^{-1}$)                                    | 104$^{a}$ | 98$^{a}$ | 90$^{b}$ | 106$^{a}$ | 100$^{a}$ | 100.20$^{a}$ | 0.03     | 13       |
| TBARS$^{2}$ (µmol g$^{-1}$)                                 | 7.53$^{a}$ | 14.79$^{a}$ | 30.57$^{a}$ | 10.93$^{a}$ | 6.62$^{a}$ | 5.9$^{a}$ | 0.035    | 12       |

$^{*}$Treatments: Soy oil (SO 3%), linseed oil (LO 3%), freshwater fish residue oil (FRO 3%), soy oil and sesame oil (SO/SEO, 1.5%/1.5%), linseed oil and sesame oil (LO/SEO, 1.5%/1.5%), freshwater fish residue oil and sesame oil (FRO/SEO, 1.5%/1.5%). $^{1}$Residual Standard Deviation (RSD), $^{ns}$not significant (P<0.05). $^{2}$Means with different letter in the same line represent significant differences (p<0.05). $^{3}$Diet consumption (DC), apparent feed conversion (AFC), weight gain (WG), specific growth rate (SGR), protein efficiency rate (PER), dry matter (DM), crude protein (CP), mineral matter (MM), ethereal extract (EE), crude energy (CE), muscular glycogen (MG), liver glycogen (LG), thiobarbituric acid reactive substances (TBARS).
probable high quantity of PUFA n-3 in the hepatic tissues. Lower quantity of PUFA n-3 in the diets reduces fish susceptibility to these oxidative reactions. This fact has been demonstrated in a seabass *Lateolabrax japonicus* study, in which the fish were fed with diets that contained palm oil associated with fish oil and presented lower malondialdehyde levels (MDA) (GAO et al., 2012). The ingested PUFAs are stocked in the liver and are susceptible to peroxidation due to the activation of the peroxisome proliferator-activated receptor (PPARα) that produces hydrogen peroxide and causes tissue damages. Thus, liver is the first receptor (PPARα) that produces hydrogen peroxide and causes tissue damages. Thus, liver is the first organ susceptible to stress oxidative reactions and, consequently, lipid peroxidation (TAKAHASHI et al., 2002). In this research, SEO had a positive effect only on/when combined with LO.

Lipid profile of oils as shown in the lipid profile of the diets, changed significantly the saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids profiles of lambari, especially, the C14:0, C16:0, C16:1, oleic acid (C18:1n-9), linoleic acid (LA), linolenic acid (LNA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Table 4). The addition of SEO increased the MUFA levels in fish fed the SO and LO diets. This oil is considered a source of MUFA since it contains about 46% in the lipid profile (KOCHHAR, 2002). However, the same tendency was not observed for the diets FRO combined or not with SEO due to the similar MUFA quantities in these diets composition (Table 1). The higher desaturation rates of LNA to HUFA n-3 were observed in fish that were fed with SEO in the diets.

| Fatty acids | SO | LO | FRO | P-value | Without | With | P-value | RSD |
|-------------|----|----|-----|---------|---------|------|---------|-----|
| C12:0       |    |    |     |         |         |      |         |     |
| C16:0       | 0.08 | 0.05 | 0.06 | 0.53\* | 0.07 | 0.06 | 0.72\* | 0.06 |
| C18:0       | 20.03\* | 19.45\* | 21.12\* | 0.001 | 20.38 | 20.01 | 0.11 \* | 0.34 |
| C18:1n-9 (trans) | 0.06 | 0.09 | 0.11 | 0.53\* | 0.1 | 0.08 | 0.51 \* | 0.05 |
| C18:3n-6 | 0.25 \* | 0.19\* | 0.31\* | 0.023 | 0.26 | 0.25 | 0.80 \* | 0.04 |
| C20:2n-6 | 0.19 | 0.22 | 0.28 | 0.06\* | 0.23 | 0.23 | 0.94 \* | 0.04 |
| C20:3n-3 | 0.00\* | 0.12\* | 0.00\* | 0.002 | 0.05 | 0.03 | 0.35 \* | 0.03 |
| C22:6n-3 (DHA) | 0.69\* | 1.00\* | 0.73\* | 0.02 | 0.85 | 0.77 | 0.25 \* | 0.12 |
| SFA | 29.95\* | 28.94\* | 30.88\* | 0.003 | 30.07 | 29.78 | 0.31 \* | 0.46 |
| PUFA\’n-6/PUFA\’n-3 | 9.58\* | 2.62\* | 9.26\* | <0.0001 | 6.15\* | 8.14\* | 0.003 | 0.71 |
| HUFA\’n-3/LNA | 0.75\* | 0.32\* | 1.07\* | <0.0001 | 0.64\* | 0.80 \* | 0.03 | 0.09 |

Table 4 - Profiles of eviscerated fishes, describing main fatty acids, expressed in g/100g of total fatty acids.

*\( ^1 \) Treatments: Soy oil (SO 3%), linseed oil (LO 3%), freshwater fish residue oil (FRO 3%), soy oil and sesame oil (SO/SEO, 1.5%/1.5%), linseed oil and sesame oil (LO/SEO, 1.5%/1.5%), freshwater fish residue oil and sesame oil (FRO/SEO, 1.5%/1.5%). *\( ^2 \) Residual Standard Deviation (RSD). *\( ^3 \) not significant (P<0.05). *\( ^4 \) Means with different letter in the same line represent significant differences (P<0.05).

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Also, it was verified that the SEO efficiently promotes DHA formation in fish fed with diets with reduced LNA levels (Table 4).

The probable higher desaturation of LNA to HUFAn-3 and the formation of DHA could be related to the presence of the bioactive compounds in SEO, such as sesamin. The addition of sesamin in diets containing linseed and sunflower oil modified significantly lipid metabolism and increased the DHA quantities in the white muscle of rainbow trouts *Oncorhynchus mykiss* and hepatocytes of Atlantic salmon *Salmo salar* (TRATTNER et al., 2008a, 2008b) hepatocytes were incubated without or with a mixture of sesamin and episesamin in order to test for possible effects on lipid metabolism. Sesamin/episesamin exposure (0.05 mM, final concentration. This compound also increased the activity of enzymes related to β mitochondrial and peroxisomal oxidation, by activating PPARα in rats, and fish as the rainbow trout *Oncorhynchus mykiss* and *Lates calcarifer* (ASHAKUMARY et al., 1999; TRATTNER et al., 2008a; ALHAZZAA et al., 2012).

**CONCLUSION**

The inclusion of sesame oil increased the DHA levels in fish tissues verified by the higher rates of desaturation of LNA to HUFAn-3, improving the lipid quality and promoting the oxidative stability of hepatic tissue, without altering the growth performance and fish health.

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