

## Fatty acid composition of fillets of silver catfish fed on sunflower oil and linseed oil

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### Abstract

The use of lipids in diets for fish is an important source of essential fatty acids, which influence the presence of these fatty acids in the body of the fish. The objective of this study was to investigate the growth and composition of fatty acids in fillets of silver catfish (*Rhamdia quelen*), which were fed on a diet based on sunflower oil and linseed oil as sources of essential fatty acids. During the first 20 days of the experiment the silver catfish that were fed with linseed oil presented lower weight gain and specific growth rate, but in the last 20 days this group showed higher weight gain and specific growth rate. The diet consisting of linseed oil resulted in a lower deposition of fat in the fillets, increasing the amount of n-3 fatty acids in the flesh of the silver catfish. Although the primary n-3 fatty acid found in linseed oil is 18:3n-3, which is a medium chain fatty acid, dietary supplementation with linseed oil also resulted in an increased amount of long-chain n-3 fatty acid in the fillets (DHA: 22:6n-3), indicating that silver catfish is able to elongate the 18:3 n-3.

### Keywords

*Rhamdia quelen*

Essential fatty acids

Vegetable oil

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### Introduction

The silver catfish (*Rhamdia quelen*) is the most cultivated native fish species in southern Brazil (Baldisserotto, 2009). It is widely accepted in the market and its production characteristics include rusticity, easy handling and rapid growth, even in the winter months (Justi *et al.*, 2003; Tronco *et al.*, 2007). It has omnivorous feeding habits with a tendency to be an insectivore Gomiero, Souza and Braga (2007) or carnivore Kütter, Bemvenuti and Moresco (2009) and it is a species that accepts an inert diet from the start of exogenous feeding, which facilitates breeding.

The importance of lipids in nutrition fish is very emphasized and a wide variety of sources of vegetable or animal origin are used in the formulation of diets. Lipids are used as a source of energy and essential fatty acids (EFA). In general, if the diet to meet EFA requirements, the proper growth of the fish is achieved. Freshwater fish such as surubins have high requirements in polyunsaturated fatty acids of the omega series 3 and 6 (Losekann *et al.*, 2008).

The use of lipids in the diet of fish is an important source of essential fatty acids (EFA), which exert influence on the presence of these fatty acids in the body of the fish (Kaushik 2004; Vargas *et al.*, 2008). Therefore, the use of oils as a source of lipids makes it possible to decrease the amount of protein and costs

of feed, as well as providing essential fatty acids, which are necessary for the proper development of the fish (Martino *et al.*, 2002). Furthermore, there is a need to reduce the use of fish oil in diets using substitutes for vegetable origin. These ingredients should provide adequate amount of polyunsaturated fatty acids (omega 3) in the final product for human consumption (Losekann *et al.*, 2008).

In the breeding of Siluriformes the main sources of vegetables used as substitutes for fish oil are corn oil Martino *et al.* (2002), soybean oil Losekann *et al.* (2008) and sunflower oil (Hoffman and Prinsloo, 1995). With respect to silver catfish fry, a study by Melo, Radunz Neto and Silva (2001) showed that the level of lipids strongly influenced growth and carcass yield, and also increased the amount of fat that was deposited. Thus, the aim of this study was to evaluate the growth and composition of fatty acids in silver catfish fillets fed on a diet based on linseed and sunflower oil acids as sources of essential fatty acids.

### Materials and Methods

The experiment was conducted in the Physiology Laboratory of the Department of Physiology at the Federal University of Santa Maria in the city of Santa Maria, Rio Grande do Sul, Brazil. One hundred and twenty silver catfish fry were used (initial:  $73.0 \pm 8.8$

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Table 1. Composition of feed produced in the growth experiment with silver catfish

Ingredients	%	
	Sunflower	Linseed
Meat and pig bone flour	15	15
Soybean meal	40	40
Wheat bran	22	22
Ground com (grains)	13.99	13.99
Sunflower oil	4	-
Linseed oil	-	4
Vitamin and mineral mixture <sup>1</sup>	2	2
Choline <sup>2</sup>	1	1
Dicalcium phosphate	1	1
Salt (NaCl)	1	1
BHT <sup>3</sup>	0.01	0.01
Lysine <sup>4</sup>	0.08	0.08
Methionine <sup>4</sup>	0.18	0.18
<b>Calculated composition</b>		
Crude protein	28.92	28.92
Digestible energy <sup>5</sup> (kcal/kg)	2968.11	2968.11
Ether extract	8.10	8.10
Crude fiber	3.86	3.86
Starch	26.75	26.75
Mineral matter	6.12	6.12
Lysine	1.59	1.59
Methionine	0.43	0.43
Methionine + cysteine	0.89	0.89
Threonine	1.16	1.16
Tryptophan	0.36	0.36
Valine	1.35	1.35
Isoleucine	1.25	1.25
Leucine	2.16	2.16
Phenylalanine	1.39	1.39
Histidine	0.70	0.70
Arginine	2.10	2.10

<sup>1</sup>Vitamin and mineral mixture (per kilo of product/MIGPLUS®): folic acid, 3.000 mg; pantothenic acid, 30.000 mg; Co, 20 mg; Cu, 2.000 mg; Fe, 30.000 mg; I, 900 mg; Mn, 5.000 mg; Se, 100 mg; vit. A, 10.000.000 UI; vit. B1, 8.000 mg; vit. B2, 10.000 mg; vit. B6, 8.000 mg; vit. B12, 20.000 mg; vit. C, 20.000 mg; vit. D3, 2.000.000 UI; vit. E, 150.000 UI; vit. K3, 6.000 UI; Zn, 20.000 mg. <sup>2</sup>Choline (per kilo of product/MIGPLUS®): choline, 800.000 mg; <sup>3</sup>Butylated hydroxytoluene (BHT) – antioxidant; <sup>4</sup>Synthetic amino acids. <sup>5</sup>Calculated digestible energy.

g;  $19.2 \pm 0.3$  cm). The fish were distributed in six 250 L tanks with open system and individual water supply, at a stocking density of 20 fish/tank ( $5.84 \text{ kg m}^{-3}$ ) with daily renewal of 80% of the water volume. Oxygenation was maintained by three aerators of 20 W/tank. The design was completely randomized with two treatments and three replications. The water temperature ( $23.2 \pm 0.1^\circ\text{C}$ ) and dissolved oxygen (above 6.5 mg/L) were measured daily (9:00 am) with an oximeter (YSI 55/12FT). The pH ( $7.12 \pm 0.1$ ) was measured at the same time with a DMPH-2 (Digimed) pH meter in accordance with (Baldisserotto and Radunz Neto, 2004).

#### Diets

Two diets were tested, one based on sunflower oil and the other based on linseed oil (Table 1). The dry feed raw material was crushed, sieved and mixed with sunflower oil and linseed oil and then pelleted in a meat grinder. Drying was performed in an

oven at  $55^\circ\text{C}$  for 48 h. After drying, the feeds were stored under refrigeration. The determination of the composition of the feed was carried out according to (AOAC, 2005). For supplying the fish, the pellets were crushed and sieved to have a grain size proportional to the size of the mouth of the animals ( $\pm 1$  mm). The feed was provided to the fish once a day, at 8:00 am (3% of total biomass). This was performed using feeders located within each tank/net where the fish were able to feed and to avoid the wastage of feed due to leaching.

#### Biometric

Biometric readings (overall length and weight) and adjustments to the quantity of feed that was offered were performed after 20 and 40 days (the end of the experiment). Weighing was performed using a Shumatzu balance (precision of 0.0001 g). The specific growth rate (SGR) was calculated according to Equation 1. Before any data collection, the fish

Table 2. Zootechnical parameters of silver catfish fed with sunflower or linseed oil. SGR = specific growth rate

Parameter	Diet	
	sunflower	linseed
<b>20 days</b>		
Weight gain (%/day)	1.33±0.29	0.64±0.10*
Length (cm)	20.2±0.8	20.9±1.2
SGR (%/day)	1.18±0.23	0.61±0.09*
Tank biomass (g)	1320.7±92.8	1305.5±135.2
<b>40 days</b>		
Weight gain (%/day)	0.15±0.04	0.30±0.09*
Length (cm)	20.4±0.7	20.6±0.9
SGR (%/day)	0.15±0.04	0.29±0.08*
Tank biomass (g)	1357.7±14.0	1508.5±134.6

\* Significantly different from diet containing sunflower oil (P<0.05).

were anesthetized with Eugenol (20 mg/L of water) (Cunha *et al.*, 2010).

$$\text{SGR} = ((\ln Fw - \ln Iw)/\text{time}) \times 100 \quad (1)$$

Where Fw = final weight, Iw = initial weight, and the time in days.

The fat in the fish fillets was extracted using chloroform and methanol (Bligh and Dyer, 1959); 0.02% butylated hydroxytoluene was added to the chloroform to prevent lipid oxidation both during and after extraction. Both the oils and the fat that were extracted from the fillets were saponified in methanolic KOH solution and then esterified in methanol solution of H<sub>2</sub>SO<sub>4</sub>. The esters of the fatty acids were analyzed using a gas chromatograph (Agilent Technologies HP 6890) with a HP-INNOWax capillary column (polyethylene glycol, 30 m x 0.25 mm x 0.25 µm) and a flame ionization detector. The injector temperature was set at 250°C and nitrogen was used as carrier gas (0.9 mL min<sup>-1</sup>). After injection (1 µL, ratio 50:1), the furnace temperature was increased from 120°C to 200°C at 20°C min<sup>-1</sup>. The temperature was maintained at 200°C for 9 min and then increased from 200°C to 250°C at 10°C min<sup>-1</sup> and maintained at this temperature for 10 min. Standards of methyl esters of fatty acids (Sigma, St Louis, USA) were used for identification and the results were expressed as a percentage of the total area of the identified fatty acids.

#### Composition Chemical

The analyses of protein, moisture, lipids, ash and carbohydrates were determined according to the AOAC (2005). Moisture analysis was performed by oven drying at 105°C to constant weight. Determination of ash was performed by incineration in a muffle furnace at 550°C and protein was determined using the Kjeldahl method. The total lipids were obtained by extraction of the ethereal using Soxhlet apparatus. The levels of carbohydrates

were obtained by calculations of the difference between the other fractions.

#### Statistics

The data related to zootechnical parameters and chemical composition showed a normal distribution and was compared using Student's t-test. The fatty acid composition was tested by using the Mann-Whitney test. The data were expressed as mean ± SD (n = 3) and the minimum level of significance was 95% (p < 0.05).

#### Results and Discussion

During the first 20 days of the experiment the silver catfish fed with linseed oil had a lower weight gain and specific growth rate, but in the last 20 days this group had a higher weight gain and specific growth rate. The other zootechnical parameters were not altered by the addition of linseed oil (Table 2). It seems as if there was a period of adaptation to the diet containing linseed oil, during which growth was reduced. Previous studies with silver catfish fed on 1.7 to 5% linseed oil for 30 days revealed no changes in growth compared to fish fed with equal proportions of corn oil or fish oil (Vargas *et al.*, 2008b).

The fish fed on a diet containing sunflower oil had higher lipid and protein deposition and lower moisture content compared to the fish fed on a diet containing linseed oil (Table 3). This was probably due to the better ratio between omega 3/omega 6 that was present in the linseed oil. The ash content was not affected by the oil sources that were tested (Table 3). The fish fed on a diet containing sunflower oil showed high concentrations of LA (linoleic acid) and oleic acid (Table 4). This result can be explained by the high lipid content of these animals, in which oleic fatty acid is dominant in the adipose tissue. Similarly, a study by Ng, Lim and Boey (2004) found high levels of these fatty acids in the muscles of *Clarias gariepinus* fry that were fed a diet of 10% sunflower

Table 3. Chemical composition of silver catfish fillets fed on a diet containing sunflower oil or linseed oil for 40 days

Diet	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	Carbohydrates (%)
Sunflower	79.37±0.02	17.57±0.01	1.23±0.02	1.31±0.02	0.52±0.01
Linseed	79.43±0.02*	17.42±0.02*	1.17±0.02*	1.31±0.02	0.67±0.03

\*Significantly different from diet containing sunflower oil (P<0.05).

Table 4. Composition of fatty acids (g/100 g of total fatty acids) of oils and of fillets of silver catfish fed for 40 days on diets containing linseed oil or sunflower oil

Fatty acids	Oils		Fish	
	Sunflower	Linseed	Sunflower	Linseed
14:0	Nd	Nd	0.93 ± 0.11	0.78 ± 0.40
16:0	6.67 ± 0.02	7.07 ± 0.07	19.98 ± 0.51	20.97 ± 0.66*
18:0	4.05 ± 0.04	5.68 ± 0.06	8.52 ± 0.32	9.48 ± 0.52*
24:0	0.25 ± 0.01	Nd	0.79 ± 0.26	1.01 ± 0.16
ΣSFA	11.13 ± 0.25	12.96 ± 0.14	30.38 ± 0.58	32.49 ± 0.85*
16:1n7	Nd	Nd	2.50 ± 0.58	2.76 ± 0.66
18:1n9	27.08 ± 0.09	22.33 ± 0.29	28.85 ± 0.95	27.41 ± 0.61*
20:1n9	Nd	Nd	1.05 ± 0.04	0.95 ± 0.25
ΣMUFA	27.21 ± 0.26	22.33 ± 0.29	32.77 ± 1.25	31.52 ± 1.02
18:2n6	60.35 ± 0.50	14.68 ± 0.16	24.26 ± 1.15	17.77 ± 0.59*
18:3n3	0.58 ± 0.03	49.82 ± 0.01	0.93 ± 0.14	5.85 ± 1.25*
18:3n6	Nd	Nd	1.43 ± 0.13	0.57 ± 0.33*
20:3n3	Nd	Nd	2.58 ± 0.39	3.06 ± 0.20*
20:3n6	Nd	Nd	2.80 ± 0.19	1.85 ± 0.14*
22:2n6	Nd	Nd	1.15 ± 0.23	1.00 ± 0.08
22:6n3	Nd	Nd	3.53 ± 1.02	5.50 ± 0.44*
ΣPUFA	61.66 ± 0.5	64.71 ± 0.16	36.84 ± 1.46	36.00 ± 1.69
UFA/SFA	7.99 ± 0.20	6.71 ± 0.08	2.29 ± 0.06	2.08 ± 0.08*
Σn-6	60.35 ± 0.50	14.68 ± 0.16	29.64 ± 1.35	21.19 ± 0.67*
Σn-3	1.31 ± 0.02	50.03 ± 0.00	7.20 ± 1.22	14.80 ± 1.11*
n-6/n-3	46.02 ± 0.15	0.29 ± 0.00	4.22 ± 0.78	1.44 ± 0.08*

Results are means ± SD (n = 2 for the oils and n = 6 for the fish). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; Nd: not detected. The fatty acids 15:1; 20:0; 20:5n3 and 22:5n3 were found in lower proportions than 0.5% and did not differ between the treatments, for this reason they are not shown in the table.

oil.

In the present study, the fish fed on linseed oil had higher levels of saturated fatty acids than unsaturated fatty acids (Table 4). Similar results were found by Francis *et al.* (2006) in Murray cod (*Maccullochella peelii*) fed on a diet containing 30% linseed oil. The diets used in the present study did not significantly alter the concentration of EPA in the silver catfish but there was a higher DHA content (22:6n-3) in the body composition of the fish fed with linseed oil (Table 4). However, Vargas *et al.* (2008a) found that silver catfish fed on corn oil and linseed oil showed a decrease in DHA and EPA levels in their body composition.

The ability of silver catfish to desaturate and elongate fatty acids was evidenced by the DHA values recorded in the fish which only consumed a diet with linseed oil. Studies by Zheng *et al.* (2005) and Martino *et al.* (2002) demonstrated important

elongation and desaturation activities when C18 precursors (linoleic and linolenic acid) were incorporated into the diet. Consistent with those results, the present study found increases in the concentrations of polyunsaturated fatty acids in the body composition of silver catfish which correlated with increased amounts of these polyunsaturated fatty acids in the diet. The concentration of arachidonic acid (Table 3) was less than 0.5% in both the linseed oil and sunflower oil diets, which can be explained by the high concentration of n-3 polyunsaturated fatty acids in the diet containing linseed oil, which may have inhibited the metabolism of the n-6 series (Horrobin, 1991). Bell *et al.* (1993) observed the same behavior in Atlantic salmon (*Salmo salar*) fed on diets containing linseed oil.

The n-3:n-6 ratio in the body composition of the silver catfish was strongly influenced by diet and

the highest value was found for the diet containing linseed oil. A study by Martino *et al.* (2002) of catfish (*Pseudoplatystoma coruscans*) found similar results. The n-3:n-6 ratio obtained in the present study for the treatments with linseed oil and sunflower oil were greater than those proposed as minimum values (n-3:n-6  $\geq$  0.25) by the World Health Organization to prevent coronary heart disease (FAO/WHO, 1993).

## Conclusions

The diet containing linseed oil resulted in a lower deposition of fat in the fillets, increasing the amount of n-3 fatty acids in the flesh of the silver catfish. Although the primary n-3 fatty acid found in linseed oil is 18:3n-3, which is a medium-chain fatty acid, supplementation of the diet with linseed oil also resulted in an increased amount of long-chain n-3 fatty acids in the fillets (DHA: 22:6n-3), indicating that the silver catfish can elongate 18:3n-3 acid.

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