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Vegetable oil sources in diets for freshwater angelfish (*Pterophyllum scalare*, Cichlidae): growth and thermal tolerance

[*Fontes de óleos vegetais em dietas para acará-bandeira (Pterophyllum scalare, Cichlidae): crescimento e tolerância térmica*]

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ABSTRACT

The influence of fatty acid composition of the diets on the productive performance and on cold and heat tolerance of juvenile freshwater angelfish (*Pterophyllum scalare*), in three different phases, was studied. Phase I studied the productive performance of freshwater angelfish in a completely randomized experimental design with four treatments, canola, linseed, olive and soybean oils and four replicates during 50 days using 192 fish in 16 aquaria. Phase II studied the cold tolerance of juvenile freshwater angelfish using 72 juvenile freshwater angelfish, coming from phase I and maintained in 12 aquaria climatized chamber. The temperature was reduced 1 °C per day, until the observation of 100% fish mortality. Phase III, it was studied the heat tolerance of juvenile freshwater angelfish employing an identical procedure to phase II, but with a daily increase of 1°C. Significant differences (P>0.05) were not observed for any parameters evaluated. Thus, it was concluded that the type of vegetable oil (canola, linseed, olive and soybean) used as a diet supplement did not affect the productive performance, nor the tolerance to cold and heat, of juvenile freshwater angelfish.

Keywords: ornamental fish, fatty acids, growth, survival, temperature

RESUMO

Avaliou-se a influência da suplementação de lipídeos na dieta, com diferentes composições de ácidos graxos, sobre o desempenho produtivo e tolerância ao frio e ao calor de juvenis de acará-bandeira (Pterophyllum scalare). *O experimento foi realizado em três fases. Na fase um avaliou-se o desempenho produtivo dos peixes em delineamento inteiramente ao acaso com quatro tratamentos – óleos de canola, linhaça, oliva e soja – e quatro repetições, durante 50 dias usando 192 peixes distribuídos em 16 aquários. Na segunda fase, avaliou-se a tolerância ao frio, usando 72 peixes, procedentes da fase um, distribuídos em 12 aquários e mantidos em câmara climatizada. A temperatura foi reduzida de 1°C por dia até a observação de 100% de mortalidade dos peixes. Na fase três, avaliou-se a tolerância ao calor com procedimentos semelhantes aos da fase dois, porém a temperatura foi elevada 1°C por dia. Não foram observadas diferenças significativas (P>0,05) para os parâmetros avaliados. Conclui-se que a suplementação de óleos vegetais nas dietas não inferiu no desempenho produtivo e na tolerância ao frio e ao calor de juvenis de acará-bandeira.*

Palavras-chave: peixe ornamental, ácidos graxos, crescimento, sobrevivência, temperatura

INTRODUCTION

Feeding is one of the main success factors in fish farming because it allows fast growth and good health to the animals. Among the diet macronutrients, lipids are important sources of energy, essential fatty acids and structural components of cell membranes (Higgs and Dong, 2000; Chou *et al*., 2001). The membranes are involved in a variety of cell functions, such as the selective uptake and release of compounds, signal transduction and storage of precursors used for the synthesis of lipid-derived hormones, and all these functions are affected by the molecules that compose the membranes (Williams, 1998). The absence of rigid

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connections between lipids and the dynamic organization of the membrane are essential for its proper functioning (Singer and Nicolson, 1972). These characteristics make the membranes extremely sensitive to physical and chemical changes in the environment (Williams, 1998).

Variations in water temperature cause changes in cell membrane organization of ectotherms (Wilmer *et al*., 2005). At low temperatures, the membrane passes from the liquid crystalline phase into the gel phase and becomes more rigid according to the high lipid viscosity, while at high temperatures membrane order is steadily decreased and become hyperfluid, with little viscosity (Williams, 1998). In both situations, the membrane functions are affected which may result in physiological losses.

To neutralize the effects of the ambient temperature variation and reestablish homeostasis, ectotherms make changes in the membrane lipid composition, especially alterations in the type and quantity of unsaturated fatty acids (Hazel and Williams, 1990).

Several studies have aimed to relate the influence of the diet lipid sources (fish and vegetable oils with different fatty acids compositions) on cold tolerance for different fish species, such as channel catfish *Ictalurus punctatus* (Fracalossi and Lovell, 1995), red drum *Sciaenops ocellatus* (Craig *et al*., 1995), milkfish *Chanos chanos* (Hsieh *et al*., 2003) and rohu *Labeo rohita* (Mishra and Samantaray, 2004). Nevertheless, there are few studies on the relations between diet lipid sources and heat tolerance for fish, in spite of the increase in the global temperature of around 0,5 °C since 1975 (Hansen *et al*., 1999; Jones *et al*., 1999), and the expectation that it will continue to increase because of several ongoing anthropogenic processes.

In general, ornamental fish are raised in small tanks and ponds, which makes them especially vulnerable to greater temperature variations, both in the day-night cycle and during the seasons of the year. Moreover, manipulation of temperature and other water parameters may represent an alternative to minimize the transmission of parasites and their impact on fish farming (Garcia *et al*., 2009). So it is necessary to evaluate their tolerance to temperature variations and mechanisms to minimize their harmful effects on fish production.

The freshwater angelfish *Pterophyllum scalare* (Cichlidae) is one of the most popular ornamental species in the world, due to its peaceful coexistence with other species, relative rusticity and the peculiar shape of its body and fins. In the productive context, the freshwater angelfish is characterized by easy adaptation to captivity conditions, good acceptance of processed food, significant market value (Fujimoto *et al*., 2006) and reproduction without the need of hormonal induction. Thus, the present study aimed to evaluate the productive performance and tolerance to cold and heat for juvenile freshwater angelfish fed with diets containing different vegetable oils sources.

MATERIAL AND METHODS

The study was carried out in three phases. The first one evaluated theproductive performance of juvenile freshwater angelfish *Pterophyllum scalare* fed with diets supplemented with different vegetable oil sources; the two following phases evaluated its tolerance to cold and heat due to the vegetal oil type included in the diet.

In phase I, in a completely randomized design, four sources of vegetable oils were evaluated: canola, linseed, olive and soybean. One hundred and ninety-two fish which came from a local ornamental fish producer were used, with average standard length of 2.56±0.16cm and average weight of 0.72±0.21g, distributed in 16 aquaria (35x30x14cm, 8 liters capacity), in the density of 12 fish/aquarium (1.5 fish/L). The aquaria were provided with constant aeration and biological filtering and the temperature was maintained by a heater and a thermostat at 27±0.5^{o}C since the interval of preferred temperature of juvenile *P. scalare* is 26.7-29.2°C (Pérez *et al*., 2003). Before the beginning of the experiment, fish were maintained during 21 days in the aquaria for adaptation and were fed a commercial diet with 36% of crude protein.

The experimental diets (Table 1) were formulated to meet the nutritional requirements of protein (Zuanon *et al*., 2006; Ribeiro *et al*., 2007) and vitamin C (Blom and Dabowski, 2000) for the species, based on the chemical composition of foods (Rostagno *et al*., 2005) and the nutrients availability for the African Cichlid Nile Tilapia *Oreochromis niloticus* (Miranda *et al*., 2000; Pezzato *et al*., 2002). The food ingredients were finely milled, mixed and moistened with water at 50±5°C and pelleted. Next, the diets were dried in a stove with forced ventilation during 24 hours at 55±5°C. After processing, the pellets were ground and screened to a granulometry proportional to the gape size of the fish (a 2-mm diameter).

Table 1. Chemical-bromatological and percentage composition of the experimental diets

Ingredient	Percentage composition Diets supplemented with different vegetal lipids			
	Canola	Linseed	Olive	Soybean
Soybean meal	41.00	41.00	41.00	41.00
Corn gluten meal	13.00	13.00	13.00	13.00
Corn meal	14.62	14.62	14.62	14.62
Wheat meal	12.00	12.00	12.00	12.00
Meat and bone meal-45	10.00	10.00	10.00	10.00
Canola oil	8.00	-	-	-
Linseed oil	-	8.00	-	-
Olive oil	-	-	8.00	-
Soybean oil	-	-	-	8.00
L-Lysine	0.10	0.10	0.10	0.10
DL- Methionine	0.20	0.20	0.20	0.20
Bicalcium phosphate	-	-	-	-
Vitamin C	0.06	0.06	0.06	0.06
Common salt	0.50	0.50	0.50	0.50
Min. and vitam. suppl.[a]	0.50	0.50	0.50	0.50
BHT[b]	0.02	0.02	0.02	0.02
Calculated Chemical-bromatological Composition [c]				
Crude Energy	4,520.64	4,520.64	4,520.64	4,520.64
Digestible energy[d]	3,576.58	3,576.58	3,576.58	3,576.58
Crude protein	34.10	34.10	34.10	34.10
Digestible protein[d]	30.73	30.73	30.73	30.73
Crude Fiber	4.05	4.05	4.05	4.05
Ether Extract	11.07	11.07	11.07	11.07
Calcium	1.59	1.59	1.59	1.59
Available phosphorus[e]	0.71	0.71	0.71	0.71
Lysine	1.47	1.47	1.47	1.47
Methionine	0.61	0.61	0.61	0.61

[a]Levels of guarantee per kilogram of the product: Vit. A, 1.200.000UI ; Vit. D3 ; 200.000UI ; Vit. E, 12.000mg ; Vit. K3, 2.400mg ; Vit. B1, 4.800mg ; Vit. B2, 4.800mg ; Vit. B6, 4.000mg; Vit. B12, 4.800mg; Folic acid, 1.200mg; Pantothenate Ca, 12.000mg; Vit. C, 48.000mg; Biotin, 48mg; Choline, 65.000mg; Niacin, 24.000mg; Iron, 10.000mg; Copper, 6.000mg; Manganese, 4.000mg; Zinc, 6.000mg; Iodine, 20mg; Cobalt, 2mg; Selenium, 20mg.
[b]Butylhydroxytoluene (antioxidant)
[c]Values calculated based on the chemical composition of foods according to Rostagno *et al*. (2005).
[d]Values calculated based on the values of energy and digestible protein of the Nile tilapia (Pezzato *et al*., 2002)
[e]Values calculated for the Nile tilapia according to (Miranda *et al*., 2000).

The feeding procedure was performed manually until apparent satiety three times a day, at 8:30a.m, 12:30p.m and 4:30p.m, during 50 days. The removal of the feces was carried out once a week through siphonage followed by tank water replenishing.

At the end of the experimental period, the fish remained unfed for 24 hours, after which the individual measurements of weight (g) and standard length (cm) were performed. The following parameters of productive performance were evaluated: survival rate (SR), weight gain (WG), length gain (LG), feed intake (FI), feed conversion rate (FCR), specific growth rate (SGR) and the body condition factor (K). Differences in productive performance were evaluated through analyses of variance (ANOVA, α = 5%).

At the end of phase I, 72 fish were used in phase II and other 72 fish in phase III of the experiment. Phase II was conducted in a completely randomized experimental design, with four treatments and three replications, using the same sources of vegetable oils of the first phase. Fish that came from phase I of the experiment were distributed in 12 aquaria (30x20x8cm, 3 liters capacity), in a stoking density of six fish/aquarium (two fish/L), with constant aeration.

The aquaria were maintained in an acclimatized chamber, equipped with a 40 W fluorescent lamp, which remained turned on from 6:00a.m. to 6:00p.m. (12h light/12h dark), controlled by a timer. The acclimatized chamber was initially regulated at 27°C for a period of three days to allow fish adaptation to the new environment. After this period, the temperature was reduced by 1°C per day through thermostat adjustments, always at 12:00a.m., until the observation of 100% fish mortality. Fish mortality occurrence was verified every 24h, at 11:00a.m.

Fish were fed every day at 5:00p.m. until apparent satiety. Feces and food remains were removed through siphonage of tank bottom once a week, followed by tank water replenishing. The comparison among treatments was carried out by analyses of variance (α = 5%) for the values of temperature in which the survival rate was 50% or lower.

Phase III was conducted under an experimental design identical to phase II, except for the change in the temperature, with daily increments of 1°C.

RESULTS

Throughout the phase I there was no fish mortality. The weight gain showed averages between 0.18 and 0.22g, the averages of length gain ranged from 0.35 to 0.38cm, and the specific growth rate between 0.44 and 0.50% / day. Rate averages for feed intake were observed ranging from 0.60 to 0.64g and feed conversion from 3.04 to 3.53. The condition factor showed averages between 3.55 and 3.71. No significant differences were observed for the productive performance parameters for juvenile freshwater angelfish fed with different vegetable oil sources (Table 2).

Table 2. Average values of survival, weight gain, length gain, feed intake, feed convertion rate, specific growth rate (SGR) and the condition factor of juvenile freshwater angelfish fed with diets containing different vegetal oil sources

Productive performance parameters	Diets supplemented with vegetal oils				
	Canola	Linseed	Olive	Soybean	CV(%)
Survival (%)	100	100	100	100	0.00
Weight gain (g)	0.19	0.22	0.18	0.21	27.58
Length gain (cm)	0.38	0.37	0.35	0.38	10.28
Feed Intake (g)	0.60	0.64	0.60	0.61	4.99
Feed conversion rate	3.18	3.14	3.53	3.04	20.45
SGR (%/day)	0.46	0.50	0.44	0.50	24.34
Condition factor	3.55	3.71	3.63	3.67	6.14

CV = coefficient of variation

With the reduction in temperature in phase II, fish remained alive up to 15°C. Mortality started to occur at 14°C temperature, achieving 100% at 12°C. The sudden fish mortality did not allow the fit of expressions of survival probability according to the reduction in temperature, nor the calculation of the values of lethal temperature (TL_{50}) for the fish in each treatment. Therefore, the temperature values in which fish survival rate was 50% or lower were used for comparison among treatments. No significant differences were observed in fish survival rate among fish fed with diets supplemented with different vegetable oil sources (Figure 1).

With the increase in temperature in phase III, fish remained alive up to 37°C. Mortality started to occur at 38°C and achieved 100% at 40°C. The sudden fish mortality did not allow the fit of expressions of survival probability according to the increase in the temperature, nor the calculation of the values of lethal temperature (TL_{50}) for the fish of each treatment. Therefore, the temperature values in which fish survival rate was 50% or lower were used for comparison among treatments. No significant differences were observed in survival rate among fish fed with diets supplemented with different vegetable oil sources (Figure 2).

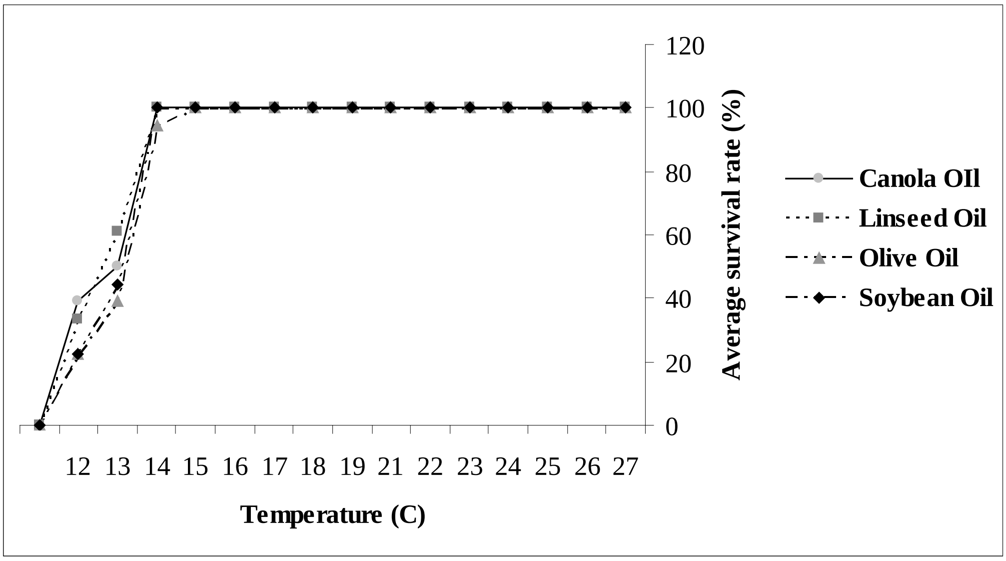

Figure 1. Relationship between water temperature decrease and the survival rate of juvenile freshwater angelfish. Values are means (n=3) for each treatment. No significant differences were observed in fish survival rate among fish fed with diets supplemented with different vegetable oil sources.

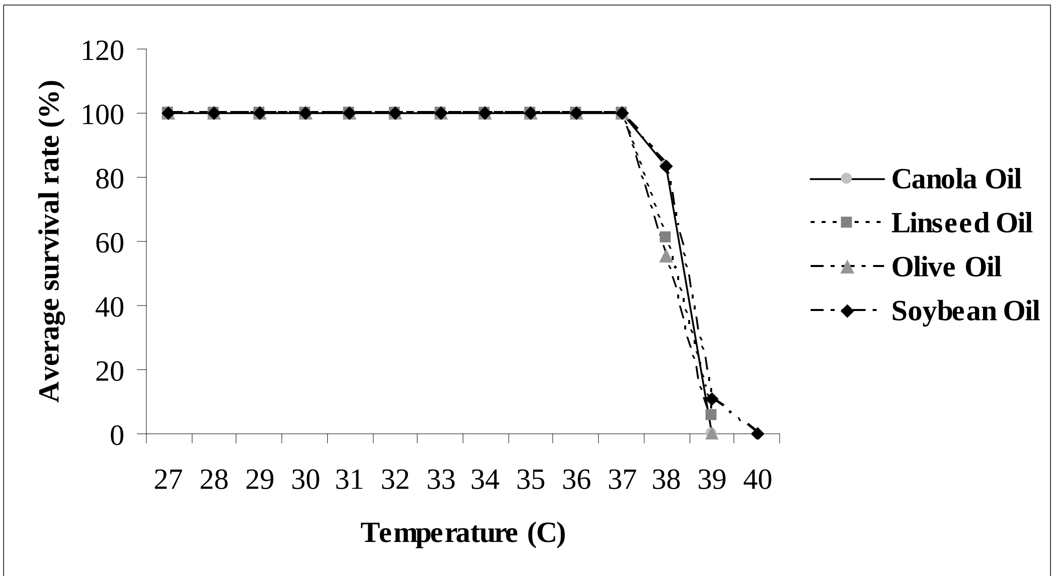

Figure 2. Relationship between water temperature increment and the survival rate of juvenile freshwater angelfish. Values are means (n=3) for each treatment. No significant differences were observed in fish survival rate among fish fed with diets supplemented with different vegetable oil sources.

DISCUSSION

The absence of significant effects of vegetable oil sources on the productive performance of freshwater angelfish may have occurred because the energy and essential fatty acids fish requirements were equally supplied by all diets. The absence of any tendency towards better results for any of the oil sources tested (Table 2) strengthens this hypothesis. Similar results were observed for the Nile tilapia (Matsushita *et al*., 2006), jundiá *Rhamdia quelen* (Losekann *et al*., 2008) and common carp *Cyprinus carpio* L. (Graeff and Tomazelli, 2007). The use of oils rich in n-3 highly unsaturated fatty acids (HUFA) has allowed improvements on the growth of juvenile Japanese sole (*Paralichthys olivaceus*) fed with a diet supplemented with squid liver oil (Kim *et al*., 2002). However, the use of these oils caused a decrease in growth in the surubim catfish *Pseudoplatystoma fasciatum* (Arslan *et al*., 2008) and African catfish *Clarias gariepinus* (Ng *et al*., 2003) fed with semi purified diets.

Another explanation for the absence of significant effects of vegetable oil supplementation on fish growth would be the use of endogenous reserves of essential fatty acids derived from the food provided before the start of the experiment (live food and/or commercial diet). In this sense, younger fish with lower lipid reserves would be more susceptible to the deficiency of these nutrients in the diet. Such hypothesis is strengthened by Uliana's *et al*. (2001) study, which observed a higher survival and growth rate in *Rhamdia quelen* larvae fed with canola oil and cod liver oil. These oils present a higher proportion of n-3/n-6 fatty acids, in comparison to the other sources of vegetable oil evaluated (soybean, corn and sunflower).

The absence of significant effects of the different sources of vegetable oils on the cold tolerance for freshwater angelfish may have occurred because freshwater fishes have great capacity to elongate and desaturate fatty acids (Moreira *et al*., 2001). Thus, the fish could increase the level of polyunsaturated fatty acids, improving the membrane fluidity, regardless of the diet consumed, since all the vegetable oil sources evaluated provide the precursors needed (linoleic and linolenic acids) for the synthesis of polyunsaturated fatty acids.

Craig *et al*. (1995) evaluated the tolerance to low temperatures for juvenile red drum fed with diets containing menhaden oil, coconut, corn and saturated fish oil. After six weeks of receiving these diets, the fish were submitted to an essay on the chronic cold tolerance, where the temperature was gradually reduced during three weeks. The authors observed that fish fed with the diet containing fish oil had the median lethal temperature significantly lower than the fish fed with the other diets. This result suggests that the high levels of polyunsaturated fatty acids of the n–3 series, and higher n–3/n–6 ratio increases the cold tolerance for red drum. However, a similar study with Nile tilapia (Atwood *et al*., 2003) did not show significant differences for cold tolerance, in spite of the profile changes in the body fatty acids observed.

Another possibility to explain the absence of a significant effect of the vegetable oil supplementation is the fast decrease in the water temperature employed by us, which may have hindered fish acclimatization. The immediate effect of the reduction in temperature is the decrease in the fish metabolism, with a reduction in the activity of all enzymes (Schmidt-Nielsen, 1997), including the desaturases needed for the homeoviscous adaptation (Snyder and Hennessey, 2003). Therefore, the slowest adaptation to cold demands some weeks (Baldisseroto, 2002) for enzymatic adaptation to occur, with the production of more enzymes and/or new isoforms adapted to low temperatures. Although the activity of the desaturase and elongase enzymes is higher in fish adapted to lower temperatures (e.g. rainbow trout; Tocher *et al*., 2004), the fast and steady reduction in temperature may have caused a harmful effect on the fish before they could provide the enzymatic change and, consequently, the homeoviscous adaptation.

In the present study, the heat tolerance for juvenile freshwater angelfish was similar to that observed by Pérez *et al*. (2003), who evaluated the critical thermal maxima (CTmax) for juvenile freshwater angelfish acclimatized at 20, 24, 28 and 30 °C, which presented values of CTmax of 36,9; 37,6; 40,6 and 40,8°C, respectively. The mentioned authors consider the end point of CTMax as the pre-death thermal point, when fish loses the capacity to escape the conditions which may lead to its death.

The expected changes in membrane lipid composition to offset the direct effects of high temperatures are the decrease in the unsaturated/saturated fatty acids ratio (Carey and Hazel, 1989) and/or the increase in the cholesterol content (Crockett, 1998). Thus, the absence of significant effects of different vegetable oil sources on heat tolerance for juvenile freshwater angelfish may be due to the fact that fish are able to synthesize saturated fatty acids and cholesterol, regardless of the supply in the diet (Tocher, 2003).

CONCLUSIONS

The type of vegetable oil (canola, linseed, olive and soybean) used as supplement in the diet did not affect the juvenile freshwater angelfish's productive performance, nor it's tolerance to cold and heat.

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