



Growth performance of *Astyanax altiparanae* fed with plant and/or animal lipid sources

Dinámica de crecimiento de *Astyanax altiparanae* alimentado con fuentes de lípidos de plantas y / o animales

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ABSTRACT

The lambari, *Astyanax altiparanae*, exhibits a great potential for aquaculture due to its omnivory, rapid growth and ease captive production. Despite of fish lipid metabolism being directly related to the dietary lipid consumed, which may lead to changes in fish growth, nothing much have been established regarding the lipid sources that can be applied in *A. altiparanae* captive production. Thus, this present research was conducted aiming to evaluate the growth performance and whole body composition of *A. altiparanae* fed with lipid sources of plant and/or animal origins. Were used a Completely Randomized design experiment with five treatments. The treatments consisted of isoproteic and isoenergetic diets, containing the following lipid sources: T1: linseed, chia and sunflower oils; T2: linseed and corn oils; T3: linseed, chia, corn and sunflower oils; T4: sunflower, corn and fish oils; T5: linseed, chia, sunflower, corn oils and bovine fat. Each treatment was replicated six times, where the experimental units consists of 10 fishes (averaged weight: 4.0 + 0.5 g) placed in an aquarium containing 80L of dechlorinated water. Were compared the growth performance parameters among the treatments by applying an one-way analysis of variance (ANOVA) at 5% significance ($P < 0.05$). The results revealed that both growth performance and whole body composition of *A. altiparanae* were not affected by the lipid source, which indicate that these fishes can efficiently use both vegetable lipid sources as well as mixtures of vegetable and animal lipid sources without any growth disadvantages.

Keywords: bovine fat, fish nutrition, neotropical fish, vegetable oils.

RESUMEN

El lambari, *Astyanax altiparanae*, exhibe un gran potencial para la acuicultura debido a su crecimiento omnívoro, rápido y fácil de cautiverio. A pesar de que el metabolismo de los lípidos en los peces está directamente relacionado con los lípidos de la dieta, lo que puede conducir a cambios en el crecimiento de los peces, no se ha establecido mucho sobre las fuentes de lípidos que se pueden aplicar en la producción en cautiverio de *A. altiparanae*. Por lo tanto, esta investigación se realizó con el objetivo de evaluar el rendimiento del crecimiento y la composición del cuerpo de *A. altiparanae* alimentados con fuentes de lípidos de origen vegetal y animal. Se utilizó un experimento de diseño completamente al azar con cinco tratamientos. Los tratamientos consistieron en dietas isoproteicas e isoenergéticas, que contenían las siguientes fuentes de lípidos: T1: aceites de linaza, chía y girasol; T2: aceites de linaza y maíz; T3: aceites de linaza, chía, maíz y girasol; T4: girasol, maíz y aceites de pescado; T5: linaza, chía, girasol, aceites de maíz y grasa bovina. Cada tratamiento se repitió seis veces, las unidades experimentales consisten en 10 peces (peso promedio: 4.0 + 0.5 g) colocados en un acuario que contiene

80 litros de agua desclorada. Se compararon los parámetros de rendimiento de crecimiento entre los tratamientos mediante la aplicación de un análisis de varianza unidireccional (ANOVA) con una significancia del 5% ($P < 0.05$). Los resultados revelaron que tanto el rendimiento del crecimiento como la composición del cuerpo de *A. altiparanae* no se vieron afectados por la fuente de lípidos, lo que indica que estos peces pueden usar eficientemente tanto fuentes de lípidos vegetales como mezclas de fuentes de lípidos vegetales y animales sin ninguna desventaja de crecimiento.

Palabras clave: Grasas bovinas, nutrición de peces, peces neotropicales, aceites vegetales

INTRODUCTION

Among the Neotropical fish species, the lambari, *Astyanax altiparanae*, has been highlighted as one of the most relevant species for the Latin American aquaculture (Campelo *et al.*, 2018) as its production has served not only for human consumption but also for the live bait market. The omnivorous feeding habit of *A. altiparanae* also favors its captive breeding. Furthermore, the *A. altiparanae* has several advantages (e.g., rapid growth, short life cycle, ease of captive production associated with its small size) that contribute to this species potential as an experimental organism model (Pontes *et al.*, 2019), which means that studies conducted with *A. altiparanae* can support the production of other fish species of commercial interest, especially those of omnivorous eating habits.

Aquaculture is still dependent on ingredients from industrial fisheries such as fishmeal and fish oil (Tacon and Metian, 2008) as these ingredients are relevant sources of energy (Bell *et al.*, 2003) and rich fatty acids such arachidonic (ARA; 20: 4n6), eicosapentaenoic (EPA; 20: 5n6) and docosapentaenoic (DHA; 22: 6n3) (Ng and Wang, 2011). However, in recent years, there has been a decline in industrial fishery resources, causing fluctuations in these products' supplies and prices, which has raised discussions about potential alternative sources to replace fish oils (Bell *et al.*, 2002; Turchini *et al.*, 2010; Tacon and Metian, 2015). Among the ingredients that may replace fish oil as lipid source, the vegetable oils has presented considerable potential. However, the vegetable

oils are usually poor in fatty acids such as ARA, EPA and DHA (Paulino *et al.*, 2018).

Some tropical fish species are capable of synthesizing ARA, EPA and DHA from fatty acid precursors as the linolenic (18: 3n3) and linoleic (18: 2n6) acids (Tocher, 2015) that must be presented in their diets. However, enzymes that act on the bioconversion of fatty acids have higher affinity for n3 series fatty acids (Henderson and Tocher, 1987), which may lead to higher EPA formation compared to ARA formation (Bell and Koppe, 2010), reinforcing the idea that the diets has to present adequate dietary ratios of precursor for n3 and n6 fatty acids.

The fishes' fatty acid profiles are directly related to the profile of their consumed diets (Tocher, 2010). Alteration in dietary fatty acid profiles may influence fish lipid metabolism, which can lead to changes in growth, carcass fatty acid profile and centesimal composition (Bell *et al.*, 2002; Castro *et al.*, 2016). Thus, this study was conducted aiming to evaluate the growth performance and whole body composition of *A. altiparanae* fed on diets containing different sources of saturated and unsaturated fatty acids.

MATERIAL AND METHODS

This study was conducted at the Fish Bioclimatology and Nutrition Laboratory of the Fish Farming Sector at the Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil (20°45'S, 42°52'W) and was approved by CEUAP (2017), protocol 017/2017.

A completely randomized experimental design with five treatments and six replications was used. The treatments consisted of five isoproteic (300.46g crude protein kg⁻¹ diet) and isoenergetic (4353.4kcal crude energy kg⁻¹ diet) diets that contained different lipid sources. Were used the following treatments:

T1) linseed, chia and sunflower oils; T2) linseed and corn oils; T3) linseed, chia, corn and sunflower oils; T4) sunflower, corn and fish oils; T5) linseed, chia, sunflower, corn oils and bovine fat. The percentage composition, chemistry and fatty acid profile of the diets are shown in Table 1 and Table 2.

Table 1. Formulation and composition of experimental diets containing different lipid sources.

Ingredients (g kg ⁻¹)	Treatments				
	T1	T2	T3	T4	T5
Soybean meal	437.0	437.0	437.0	437.0	437.0
Fish meal	120.0	120.0	120.0	120.0	120.0
Corn flour	220.0	220.0	220.0	220.0	220.0
Wheat flour	85.0	85.0	85.0	85.0	85.0
Inert	4.3	4.3	4.3	4.3	4.3
Cellulose	2.5	2.5	2.5	2.5	2.5
L - Lysine	2.0	2.0	2.0	2.0	2.0
DL - Methionine	10.0	10.0	10.0	10.0	10.0
Bicalcium phosphate	26.5	26.5	26.5	26.5	26.5
Salt	2.5	2.5	2.5	2.5	2.5
Premix vit/min ¹	10.0	10.0	10.0	10.0	10.0
BHT ²	0.2	0.2	0.2	0.2	0.2
Linseed oil	40.0	20.0	30.0	0.0	5.7
Chia Oil	20.0	0.0	10.0	0.0	0,35
Sunflower oil	20.0	0.0	10.0	22.0	1.9
Corn oil	0.0	60.0	3,00	18.0	5.7
Fish oil	0.0	0.0	0.0	4.0	0.0
Bovine fat	0.0	0.0	0.0	0.0	63.2
Chemical composition (g kg ⁻¹)					
Dry matter (g kg ⁻¹)	923.8	919.9	905.4	922.2	908.7
Crude energy (kcal kg ⁻¹)	4399	4402	4069	4338	4459
Crude protein (g kg ⁻¹ of DM)	312.1	294.6	301.4	310.9	304.0
Ether extract (g kg ⁻¹ of DM)	86.9	68.1	68.1	86.4	64.1
Ash (g kg ⁻¹ of DM)	86.8	85.6	85.8	87.2	85.2

¹Warranty levels per kilogram of product (Supremais, Campinas/SP, Brazil): Vit. A = 1.200.000 IU; Vit. D3 = 200.000 IU; Vit. E = 12.000 mg; Vit. K3 = 2.400 mg; Vit. B1 = 4.800 mg; Vit. B2 = 4.800 mg; Vit. B6 = 4.000 mg; Vit. B12 = 4.800 mg; folicacid = 1.200 mg; calciumpantothenate = 12.000 mg; Vit. C = 48.000mg; Biotin = 48 mg; Choline = 65.000 mg; Nicotinicacid = 24.000 mg; Fe = 10.000 mg; Cu = 6.000 mg; Mn = 4.000 mg; Zn = 6.000 mg; I = 20 mg; Co = 2 mg; Se = 20 mg.²Butylatedhydroxytoluene (antioxidant). DM: dry matter. T1: linseed, chia and sunflower oils; T2: linseed and corn oils; T3: linseed, chia, corn and sunflower oils; T4: sunflower, corn and fish oils; T5: linseed, chia, sunflower, corn oils and bovine fat.

Table 2. Fatty acid profiles of experimental diets containing different lipid sources.

	Fatty acids (g kg ⁻¹ of total identified fatty acids)				
	T1	T2	T3	T4	T5
12:0	0.1	0.0	0.0	0.1	0.1
14:0	0.4	0.2	0.2	1.3	2.1
15:0	0.1	0.0	0.1	0.3	0.4
16:0	7.7	10.7	9.0	12.1	19.9
17:0	0.0	0.1	0.3	0.3	0.5
18:0	4.3	3.3	3.9	3.1	18.2
23:00	0.0	0.0	0.0	0.2	0.0
24:00	0.0	0.1	0.1	0.5	0.0
14:1	0.0	0.0	0.0	0.2	0.6
16:1	0.5	0.8	0.6	3.0	2.1
18:1	31.8	29.2	29.9	39.2	32.5
20:1n9	0.4	0.5	0.4	1.3	0.5
22:1n9	0.1	0.2	0.2	0.5	0.0
18:2n6	17.6	40.3	27.6	19.8	11.1
18:3n3	34.6	12.8	23.9	1.4	6.6
20:4n6 (ARA)	0.0	0.0	0.2	0.7	0.1
20:5n3 (EPA)	0.6	0.5	0.6	3.8	0.5
22:6n3 (DHA)	0.4	0.3	0.3	7.5	0.3
ΣSAFA	12.55	14.50	13.47	17.90	41.32
ΣMUFA	33.23	30.78	31.59	46.30	36.47
ΣPUFA	54.22	54.72	54.94	35.80	22.22
n3	32.34	30.03	27.23	37.00	31.16
n6	17.61	40.79	28.10	20.69	11.85
n6/n3	0.49	2.96	1.11	1.53	1.37

ΣSAFA: sum of saturated fatty acids; ΣMUFA: sum of monounsaturated fatty acids; ΣPUFA: sum of polyunsaturated fatty acids. T1: linseed, chia and sunflower oils; T2: linseed and corn oils; T3: linseed, chia, corn and sunflower oils; T4: sunflower, corn and fish oils; T5: linseed, chia, sunflower, corn oils and bovine fat.

In order to avoid potential interference of the fatty acid macro-ingredients profiles, the soybean and wheat bran, the fish- and cornmeals were degreased before experimental diets were prepared. To this, three liters of chloroform was added for each kilo of ingredient. After mixing the mixture

(3 min), it was vacuum filtered through a Büchner funnel (with filter paper) connected to a Kitasato flask. This process was repeated three times for each ingredient and the solid material was dried at room temperature. After degreasing, the macro-ingredients were milled in hammer mills (with 0.5 mm diameter

sieve) and were manually mixed with the micro-ingredients and the different oil source proportions. The diets were pelletized in a meat grinder (FILIZOLA, P-22, São Paulo, SP, Brazil), dried in a forced ventilation oven (Marconi Equipamentos para Laboratórios, MA 035, Piracicaba, SP, Brasil) at 50°C, ground in a manual mill (BOTIMETAL, Bilac, SP, Brazil) and sifted to obtain 0.8 mm pellets.

The analyzes for chemical composition and fatty acid profiles of the diets were conducted, respectively, at the Food Analysis Laboratory of the Department of Animal Science (at UFV) and at the Natural Resources Laboratory of the Institute of Ecology and Environmental Science at Republic University, Montevideo, Uruguay.

Three hundred *A. altiparanae* specimens (average weight of 4.0 + 0.5g) were distributed in 30 aquariums (80L) containing 50L of dechlorinated water, at a density of 10 fishes per aquarium. The aquariums were maintained in a 2.5L min⁻¹ flow recirculation system, and were equipped with mechanical and biological filters, constant aeration and thermostatically controlled heating. The experimental units were covered with white nylon mesh (2mm) to prevent fish escaping. The laboratory was maintained at a 12h photoperiod by using fluorescent lamps (60 watts) controlled by analog timer. The water temperature (28 ± 0.5°C) was daily measured, before each feeding, by means of a common alcohol thermometer (0 to 100°C). The pH (7.0 + 0.2) and total ammonia (0.22 + 0.20ppm) parameters were weekly measured by using colorimetric kits, and dissolved oxygen (6.5 + 0.5mg L⁻¹) measurement was achieved by an ProPlus Multiparameter Equipment (YSI incorporated, Yellow Springs, OH, USA).

The fish were manually fed for nine weeks, three times a day (8:00 am, 12:00 am and 5:00 pm) until apparent satiety. At the end of the experiment, the fish were euthanized using a clove, *Syzygium aromaticum*, essential

oil overdose (400mg L⁻¹) and weighed on an analytical balance (MB45 Toledo ® 0.01g, São Bernardo do Campo, SP, Brazil) with 0.01g accuracy to evaluate the following zootechnical variables: Weight Gain = Final Weight - Initial Weight, Specific Growth Rate = [(ln final weight - ln initial weight) X 100]/time (in days), Carcass Yield = [(final weight - offal weight) / final weight] X 100, Hepatosomatic Index = (liver weight / final weight) x 100, Viscerosomatic Index = (viscera weight / final weight) x 100, Visceral fat index = (visceral fat weight / final weight) x 100.

For the whole body composition, a fish pool from each treatment was dried in a forced ventilation oven at 60°C for 24h. The samples were processed in a ball mill (Botimetal, Bilac, SP, Brazil). By following methodologies previously described elsewhere (Detmann *et al.*, 2012), we determined the crude protein, crude energy, ether extract, dry matter and ash contents.

The obtained data were submitted to the Lilliefors test to verify the normality of the errors and the Bartlett test to verify the homogeneity of the variances. All the results were subjected to an unidirectional analysis of variance (ANOVA) with significance of 5% ($P < 0.05$), and when needed, the mean comparison test (Tukey test, $P < 0.05$) was applied. All statistical analyzes were conducted by using the estatistical software R (version 3.3).

RESULTS AND DISCUSSION

In this study, no differences ($P > 0.05$) were observed in the weight gain, specific growth rate, carcass yield and hepatosomatic, viscerosomatic and visceral fat indices in fish fed diets containing different lipid sources (Table 3). These results show that *A. altiparanae* can efficiently use lipid sources of plant and animal origin for both energy and fatty acid supplies.

Table 3. Growth performance of *Astyanax altiparanae* fed on diets containing different sources of saturated and unsaturated fatty acids.

Variables	Diets					CV (%)
	T1	T2	T3	T4	T5	
Weight gain (g) ^{ns}	0.81 ± 0.25	0.54 ± 0.09	0.55 ± 0.29	0.59 ± 0.20	0.74 ± 0.23	32.9
Specific Growth Rate (%/ dia) ^{ns}	0.31 ± 0.06	0.21 ± 0.04	0.25 ± 0.07	0.23 ± 0.07	0.29 ± 0.01	27.47
Carcass Yield (%) ^{ns}	79.38 ± 1.98	81.75 ± 1.81	81.28 ± 2.43	80.17 ± 2.27	82.0 ± 2.54	2.68
Hepatosomatic Index (%) ^{ns}	0.71 ± 0.16	0.76 ± 0.13	0.75 ± 0.18	0.75 ± 0.18	0.78 ± 0.14	26.56
Viscerosomatic Index (%) ^{ns}	16.31 ± 2.04	12.69 ± 2.05	13.19 ± 2.46	14.76 ± 2.57	15.33 ± 2.67	16.29
Visceral Fat Index (%) ^{ns}	1.24 ± 0.75	1.67 ± 0.35	1.66 ± 0.23	1.70 ± 0.42	1.76 ± 0.20	26.57

ns: not significant; CV: coefficient of variation. T1: linseed, chia and sunflower oils; T2: linseed and corn oils; T3: linseed, chia, corn and sunflower oils; T4: sunflower, corn and fish oils; T5: linseed, chia, sunflower, corn oils and bovine fat.

Since lipids are the main source of digestible energy for fishes (Lim *et al.*, 2011), the sources used in the present investigation probably supplied the *A. altiparanae* fatty acid requirements. The complete replacement of fish oil by the mixture containing soybean and linseed oils also did not affect the performance of these fishes in previous investigation (Pontes *et al.*, 2019). These findings are similar to these ones described for other fish species such as surubim (*Pseudoplatystoma coruscans*) (Martino *et al.*, 2002), panga (*Pangasius hypophthalmus*) (Asdari *et al.*, 2011), murray cod (*Maccullochella peelii peelii*) (Turchini *et al.*, 2011) and Nile tilapia (*Oreochromis niloticus*) (Ng *et al.*, 2013), where diets containing vegetable lipid sources did not negatively affect the fish growth.

Such lack of differences in the *A. altiparanae* performance fed in our experimental diets may also be associated with the omnivorous eating habits of these animals. *Astyanax altiparanae*, like most of other species belonging to the *Genus*, is an omnivorous species that can feed on algae, insects (eggs, larvae and adults) as well as fruits and seeds (Vazzoler and Menezes, 1992; Vaz *et al.*, 2000). Therefore, these fishes are well

knowing for having high feeding flexibility (Abilhoa, 2007). It is also possible that tested fishes have adapted to the different sources of lipids used, which resulted in the absence of growth losses.

Another possible explanation for our findings would be the fatty acid ratios of the n6 and n3 series of tested diets, which although composed of different lipid sources were efficient in providing the requirements for essential fatty acids for *A. altiparanae*. For investigations using other omnivorous eating habit fishes, diets containing different n6/n3 ratios were not capable of interfering in the fish production performance (Paulino *et al.*, 2018). Similar results were also observed for carnivorous species such as murray cod *Maccullochella peelii peelii* (Senadheera *et al.*, 2010) and rainbow trout, *Oncorhynchus mykiss* (Thanuthong *et al.*, 2011) when these fishes fed on diets containing different n6/n3 fatty acid ratios obtained from mixtures of vegetable oils. As demonstrated for these omnivorous/carnivorous previous fish species, our results for fish whole body compositions were unaffected by the tested diets (Table 4). However, for the same species in this study, the total replacement of fish

oil with vegetable oil mixtures altered the carcass lipid content (Pontes *et al.*, 2019). In addition, fish fed the diet containing only linseed oil had the lowest fat deposition in the carcass (Pontes *et al.*, 2019).

The use of alternative sources to fish meal and oils without harming the animals has become a fundamental tool for the aquaculture growth, especially regarding the sustainability of production. Such aspects becomes even more relevant given the sharp decline in fishery resources, which has caused fluctuations in the supply and price of both fish meal and oils (Bell *et al.*, 2002; Turchini *et al.*, 2010; Tacon and Metian, 2015).

CONCLUSIONS

A. altiparanae can efficiently use both lipid sources of vegetable origin (alone or mixed with animal-originated oils) without exhibiting any growth disadvantages.

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