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Replacement of animal protein with vegetable protein in the diets of *Astyanax altiparanae*

Fábio Rosa Sussel[1*], Elisabete Maria Macedo Viegas[2], Mariana Machado Evangelista[1], Giovani Sampaio Gonçalves[1], Fernando André Salles[1] and Ligia Uribe Gonçalves[2]

*[1]Agência Paulista de Tecnologia dos Agronegócios, Av. Três de Maio, 654, 18133-445, São Roque, São Paulo, Brazil. [2]Departamento de Zootecnia, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, São Paulo, São Paulo, Brazil. *Author for correspondence. E-mail: sussel@apta.sp.gov.br*

ABSTRACT. The aim of this study was to evaluate the effect of replacing animal protein with vegetable protein sources on the productive performance of *Astyanax altiparanae* (lambari-do-rabo-amarelo). Five experimental diets were formulated with increasing replacement levels of animal protein by vegetable protein. A total of 9000 individuals (initial mean weight 1.18 ± 0.12 g, initial mean length 2.1 ± 0.3 cm) were distributed in 20 net cages (1 m^3) with a density of 450 ind. m^{-3}. Cages were randomly placed in a pond (180 m^2, 1.5 m deep, 10% water renewal per day). After 63 days of cultivation, total count and individual biometrics from 20% of each experimental unit were taken. Mean weight, total length, survival, feed conversion, biomass weight gain and proximate body composition were evaluated. The experimental design was completely randomized with five treatments and four replications. The reduction in the formulation cost achieved by increasing levels of vegetable protein compensated the slight decrease in biomass gain. Besides that, inclusion of vegetable protein resulted in greater fat deposition, suggesting future exploration of *A. altiparanae* as a functional food.

Keywords: productive performance, nutrition, fat deposition, intensive production.

Substituição de proteína animal por proteína vegetal em dietas para lambari-do-rabo-amarelo *Astyanax altiparanae*

RESUMO. O objetivo desse trabalho foi avaliar o efeito da substituição de fontes de proteína animal por fontes proteicas vegetais no desempenho produtivo do *Astyanax altiparanae* (lambari-do-rabo-amarelo). Cinco dietas experimentais foram formuladas com níveis crescentes de substituição de proteína animal por proteína vegetal. Foi distribuído o total de 9.000 indivíduos (peso médio inicial 1,18 ± 0,12 g, comprimento médio inicial 2,1 ± 0,3 cm) em 20 tanques-rede (1 m^3; 450 ind. m^{-3}). Os tanques-rede foram aleatoriamente dispostos em um viveiro (180 m^2, profundidade: 1,5 m, renovação de água: 10% ao dia). Após 63 dias de cultivo, foi realizada a contagem total dos indivíduos e biometria individual de 20% de cada unidade experimental. Foram avaliados peso e comprimento médios, sobrevivência, conversão alimentar, ganho em biomassa e composição corporal. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e quatro repetições. A diminuição alcançada no custo de formulação pela inclusão de níveis crescentes de proteína de origem vegetal mais que compensou a ligeira diminuição no ganho em biomassa. Ademais, a inclusão de proteína vegetal resultou em maior deposição lipídica, sugerindo exploração futura de *A. altiparanae* como alimento funcional.

Palavras-chave: desempenho produtivo, nutrição, deposição lipídica, produção intensiva.

Introduction

Among different feedstuffs available worldwide for farm animals, the total included in the diets for aquatic organisms represent 3% of the total; however, this sector is responsible for the consumption of 46% of the fish meal produced (TACON, 2005). There is a widespread concern about the stagnation of world fisheries resources and great efforts are made to reduce the use of fish meal in aquatic feeds (TACON; METIAN, 2008). Plant-based ingredients are a good source to replace fish meal in aquatic feed, because they have a continuous supply and high quality nutrients (TUSCHE et al., 2012). However, plant-based protein sources commonly have low digestibility, are deficient in lysine and methionine and may have anti-nutritional characteristics (CYRINO et al., 2010; NRC, 2011).

Experiments replacing animal protein (AP) with vegetable protein (VP) showed no significant reductions in growth both in marine (KAUSHIK et al., 2004; SALZE et al., 2010) and freshwater species (THIESSEN et al., 2004; WATANABE et al., 1998).

Omnivorous fish have morphological and physiological adaptations that enable the use of diets with high percentages of vegetable ingredients, because these organisms exhibit better assimilation of carbohydrates (KUBARIK, 1997) and amino acids from vegetable sources (TENGJAROENKUL et al., 2000) compared with carnivorous fish.

Astyanax altiparanae (lambari-do-rabo-amarelo) is native to Brazil and has attracted interest as a suitable species for aquaculture; however, only a small number of studies have been conducted on its nutrition (ABIMORAD; CASTELLANI, 2011). This omnivorous species has a number of desirable traits, such as high prolificacy, easily obtained fingerling, adaptation to thermal variations and fast growth, reaching market weight (10-15 g) within approximately 3 months (GARUTTI, 2003). In Brazil, this fish is used as live bait for the sport fishing of the peacock bass (*Cichla* spp.) among other species, as well as for human consumption as an appetizer (PORTO-FORESTI et al., 2005).

Within the context of formulating diets using alternative ingredients of high quality and availability, the present study aimed to evaluate the replacement of animal protein with vegetable protein on the growth performance of *A. altiparanae*, as well as its possible effect on fish body composition.

Material and methods

Experimental diets

Five experimental diets were formulated with increasing replacement levels of animal protein sources by vegetable protein sources (Table 1). Poultry viscera meal, meat meal and marine fish meal were the sources of animal protein, added on diet VP0 at 12.97; 10.80 and 7.00% of the total diet, respectively. Levels of animal protein sources on subsequent diets (VP25, VP50 and VP75) were 75, 50 and 25% of those values of VP0. VP100 had no animal origin ingredients. The level of crude protein (CP) was the same for the five experimental diets (26% CP). The final cost was calculated taking into account the current price of each ingredient, on January 2012, in the factory where feeds were produced (CIF price, Santa Fé do Sul- São Paulo State). In addition to nutrition, technical and commercial issues were considered in the diet formulation. Feed ingredients were ground to a 0.9-mm particle size, mixed and then moistened (10% water: volume). Diets (Table 1) were extruded into 3-mm pellets using an equipment with 30 kg h^{-1} capacity (Inbramaq, Ribeirão Preto, São Paulo State, Brazil), oven dried (forced air; 40°C; 24h), stored in plastic bags and frozen (-20°C).

Table 1. Formulation and chemical composition of experimental diets with increasing levels of vegetable protein.

Ingredients (%)	VP0	VP25	VP50	VP75	VP100
Rice husk (39% fiber)	3.00	2.25	1.50	0.75	0.00
Corn (9% CP)	32.32	28.96	25.60	22.25	18.9
Soybean meal (46% CP)	0.00	6.58	13.16	19.73	26.32
Cotton meal (38% CP)	0.00	3.00	6.00	9.00	12.00
Corn gluten (60% CP)	0.00	1.25	2.50	3.76	5.00
Wheat meal (16% CP)	20.00	20.00	20.00	20.00	20.00
Broken rice (71% starch)	8.00	8.00	8.00	8.00	8.00
Poultry viscera meal (58% CP)	12.97	9.72	6.48	3.25	0.00
Meat meal (43% CP)	10.80	8.10	5.40	2.70	0.00
Marine fish meal (56% CP)	7.00	5.25	3.50	1.75	0.00
Blood meal (spray-dried, 80% CP)	4.00	3.00	2.00	1.00	0.00
Salt	0.30	0.31	0.30	0.30	0.30
Limestone (38% Ca)	1.00	1.39	1.79	2.18	2.57
Dicalcium phosphate	0.00	0.85	1.70	2.54	3.39
Soybean oil	0.00	0.73	1.46	2.18	2.91
Mold-Nil-Dry[1]	0.10	0.10	0.10	0.10	0.10
Oxinyl Dry[2]	0.01	0.01	0.01	0.01	0.01
Vit. and Min. Suplem.[3]	0.50	0.50	0.50	0.50	0.50
Cost (US$/T)	350.42	320.48	311.42	307.60	294.71
Calculated Composition (%)					
Moisture	10.34	10.35	10.35	10.36	10.36
Crude protein	26.33	26.25	26.17	26.08	26.00
Ether extract	5.86	5.65	5.43	5.22	5.00
Crude fiber	5.64	5.54	5.44	5.33	5.23
Calcium	3.18	2.89	2.59	2.30	2.00
Total phosphorus	1.58	1.48	1.38	1.28	1.18
Starch	31.98	30.99	29.99	29.00	28.00
Available phosphorus	0.80	0.80	0.80	0.80	0.80

1.Antifungal. 2. Antioxidant. 3.Vitamin and mineral supplement: Vitamin C, 300 mg; vitamin A, 12,000 IU; vitamin B1, 20 mg; vitamin B2, 20 mg; vitamin B12, 40 mcg; vitamin B6, 17.50 mg; Vitamin D3, 3,000 IU; vitamin E, 150 mg; Vitamin K3, 15 mg; calcium pantothenate, 50 mg; niacin, 100 mg; folic acid, 6 mg; biotin, 1 mg; choline chloride, 500 mg; cobalt, 0.40 mg; copper, 17.50 mg; iron, 100 mg; manganese, 50 mg; selenium, 100 mg; zinc, 120 mg; excipient q.s., 1,000 g.

Experimental procedure

The experiment was carried out at the Unidade de Pesquisa e Desenvolvimento de Pirassununga - São Paulo State, Brazil. A total of 9,000 individuals (1.18 ± 0.12 g; 2.1 ± 0.3 cm) were distributed in 20 net cages (5 mm mesh size) of $1m^3$ and a density of 450 ind. m^{-3}. The experimental units were randomly placed in a pond (180 m^2), 1.5 m deep and with 10% water renewal per day.

The experiment lasted a total of 63 days, during which, fish were fed 3 times a day to apparent satiation, with no leftovers. The water quality in the experimental pond was monitored daily for temperature (25-32°C) and dissolved oxygen (3.5-5.3 mg L^{-1}), while the pH (6.3-7.6) and total ammonia (0-2.0 mg L^{-1}) were evaluated weekly.

At the end of the experimental period, fish were fasted for 24h, counted and weighed. Individual biometrics was measured in a sample of 20% (n = 90) of the subjects from each experimental unit, which were euthanized by anesthetic overdose with benzocaine (30 mg L^{-1}), and frozen to subsequent analysis. Whole-body composition was determined in pooled samples of 20 fish per cage (80 fish per treatment). Samples of whole-body were analyzed for proximate composition, according to the procedures described by AOAC (1990).

Moisture obtained by air oven drying (105°C) for 16 hours;

Ash content by burning at high temperatures in a muffle furnace for four hours;

Crude protein by semi-micro Kjeldahl method. A factor of 6.25 was employed.

Lipid extraction by petroleum ether during six hours (Soxhlet).

The growth parameters were calculated as follow: biomass (g) weight gain (BG, %) = 100 x (total final weight – total initial weight) ÷ total initial weight; mean weight gain (WG, %) = 100 x (final mean weight$_{(g)}$ – initial mean weight $_{(g)}$) ÷ initial mean weight$_{(g)}$; feed conversion (FC) = feed intake$_{(g)}$ ÷ weight gain$_{(g)}$; survival rate (SU, %) = 100 (*n* initial of fish – *n* final of fish) and length (LT).

The experiment was a completely randomized design with five treatments (VP0; VP25; VP50; VP75; VP100) and four replications for each treatment. Data were subjected to one-way ANOVA and evaluated by a simple linear regression analysis using the PROC GLM from SAS 9. Additionally, the occurrence of significant differences among treatments was assessed by Tukey's test at the significance level of 0.05.

Results and discussion

Performance data are presented on Table 2. There were no significant differences among the treatments following increasing levels of vegetable protein on fish diets.

Table 2. Mean values of weight (WG), total length (LT), survival (SU), feed conversion (FC) and biomass gain (BG) of *A. altiparanae* fed increasing levels of vegetable protein after 63 experimental days.

Treatments	WG (g)	LT (cm)	SU (%)	FC	BG (kg)
VP0	7.14a*	7.02a	90.44a	1.38a	2.900a
VP25	7.55a	7.23a	87.17a	1.39a	2.962a
VP50	7.09a	7.13a	87.56a	1.38a	2.812a
VP75	7.01a	7.00a	88.44a	1.39a	2.787a
VP100	6.85a	7.09a	88.56a	1.43a	2.712a
S.E.M.**	0.22	0.08	1.2	0.06	0.09

*Values followed by different letters in the same column are significantly different (Tukey's test, p = 0.05). **Standard error of the mean.

However, regression analysis indicated a negative linear effect (p = 0.0461) for the replacement of AP with VP on Biomass Gain (Figure 1), according to the following expression:

BG (g) = 2.945g - 2.2VP

where:

BG: Biomass Gain;

VP: 100*(ratio of replacement of animal protein with vegetable protein).

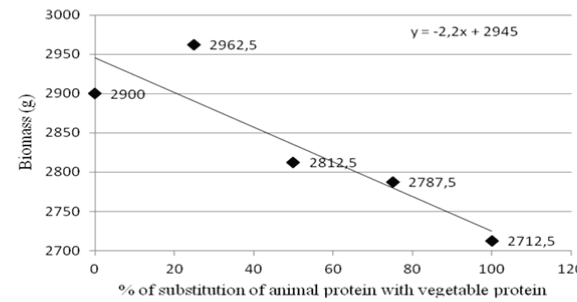

Figure 1. Effect of increasing levels of vegetable protein on Biomass Gain of *A. altiparanae* after 63 days of cultivation.

A proper analysis of the effects of changes in diet composition for fish generally cannot be made only on the basis of statistical comparisons (HUA; BUREAU, 2012). Therefore, considering the lower cost of the formula (-15.9%) of the diet with 100% VP in relation to that with 100% AP (US$ 294.71 against US$ 350.42; Table 1), it is suggested that in economic terms, the difference on production performance can be compensated by this factor.

A. altiparanae is a fish with high trophic plasticity (BENNEMANN et al., 2005). Thus, the absence of significant changes in production performance parameters for *A. altiparanae* observed in the present study may be related to the high feeding plasticity of this species, explained by the fact that omnivorous fish generally have adaptations that enable the better use of carbohydrates and proteins (TENGJAROENKUL et al., 2000). Similar results were found for others omnivorous species.

Furuya et al. (2004) evaluated the effect of the total replacement of fish meal with soybean meal on Nile tilapia (*Oreochromis niloticus*) nutrition and suggested that in diets formulated only with VP and supplemented with synthetic amino acids, it is possible to replace the fish meal with no adverse effect on the growth performance, yield or carcass chemical composition. Likewise, Lin and Luo (2011) evaluated the replacement of fish meal with soybean meal at 0, 25, 50, 75, and 100% for tilapia juvenile (*O. niloticus x O. aureus*), and found no adverse effects on growth with up to 75% replacement.

Even for carnivorous species, such as the rainbow trout, it is possible to replace the fish meal almost completely with a vegetable-based meal (WATANABE et al., 1998). In marine fish species, an almost complete replacement of AP has also already been demonstrated (KAUSHIK et al., 2004; SALZE et al., 2010).

Hua and Bureau (2012), using a meta-analysis system, studied the results of several researches regarding the replacement of AP with VP in the diet for salmonids. The authors observed that a low or moderate addition of VP (40%) replacing

AP does not affect the performance of salmonids; however, the total replacement of AP with VP causes harm, even when the diets were apparently nutritionally complete.

Taking into account that such analyses refer to carnivorous species (trout and salmon), it is suggested that for omnivorous species, which have a greater ability to use vegetable sources, the levels of VP inclusion can be higher, as observed in the present study for *A. altiparanae.*

Sales (2009) also used the meta-analysis technique to study the effect of replacing fish meal with VP concentrates on the growth of several fish species. No evidence was found supporting a trend defined for the optimum replacement level, given that replacements of up to 40% of fish meal, regardless of the protein level, do not affect growth, and for replacements above 40%, supplementation with synthetic amino acids, particularly methionine, is recommended.

In the present study, the protein content did not differ with increasing VP in the diets (Table 3). The crude protein level was around 16.00%, as also reported for *A. altiparanae* fed with 38% CP (COTAN et al., 2006) and 36% CP with different sources of oil (GONÇALVES et al., 2012).

Table 3. Mean values of the body composition of *A. altiparanae* fed diets containing a gradual replacement of animal protein (AP) with vegetable protein (VP).

Treatments	Dry matter (%)	Crude protein (%)	Ash (%)	Lipids (%)
VP0	35.05^{a*}	15.77^{a}	3.52^{a}	15.10^{bc}
VP25	35.14^{a}	16.85^{a}	3.43^{a}	15.04^{c}
VP50	36.87^{a}	17.05^{a}	3.62^{a}	16.64^{a}
VP75	36.39^{a}	16.92^{a}	3.60^{a}	15.91^{ab}
VP100	37.05^{a}	16.87^{a}	3.55^{a}	16.33^{a}
S.E.M**	0.57	0.31	0.21	0.19

*Values followed by different letters in the same column are significantly different (Tukey's test, p = 0.05). **Standard error of the mean.

However, the values of dry matter and lipids found by the cited authors differed considerably from the present study. While the mean value of dry matter in the present study was 36.1%, and of lipids was 15.80%, Cotan et al. (2006) and Gonçalves et al. (2012), observed values of 29.48 and 27.98%, respectively, for the dry matter and 10.05 and 5.62%, respectively, for lipids, and it can be explained by differences in the diet formulation.

The difference in the mean dry matter is most likely related to the fish size because higher levels of dry matter are expected in conjunction with higher levels of mineral matter within the same species for larger fish. According to Shearer (1994), in juvenile fish, the carcass (muscle, in particular) growth is greater than the growth of other body parts, and the relative sizes of the tissues and organs under appropriate nutritional conditions depend on the fish size and life cycle.

Regarding the difference in lipid levels, *A. altiparanae* with a mean weight of 7.12 g are already sexually mature, and the presence of developed gonads in females implies higher ether extract levels because, this species mobilizes large amounts of lipids for the maturation of gonads (GONÇALVES et al., 2012).

In treatments with greater amounts of VP, higher lipid levels were observed (Table 3). Lipids are a good source of digestible energy for fish (TOCHER, 2003), but the digestibility can be affected by the fatty acid composition (MENOYO et al., 2003). Martins et al. (2009) verified that the use of poultry fat as the main lipid source in diets for Atlantic halibut resulted in low overall fatty acid digestibility. Therefore, the fatty acid composition of animal ingredients (poultry viscera meal, meat meal, blood meal) present at higher concentration in VP0 and VP25 formulations may have decreased the digestibility of lipids, and it can reflect the lower lipid concentration of fish carcasses that fed these diets.

According to Gonçalves et al. (2012), *A. altiparanae* is able to desaturate and elongate F-18 carbon fatty acid chains (long chain fatty acids), producing arachidonic acid (AA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). EPA and DHA are the main constituents of the omega-3 family of essential fatty acids, which play important roles in the cell membrane structure and in metabolic processes. These fatty acids have been the focus of numerous studies over the last several decades, which suggest their various functions in the human body (MARTIN et al., 2006). However, the human body is unable to synthesize these fatty acids, thus depending on their exogenous sources. Based on these results and on the data from Gonçalves et al. (2012) and the ability of fish to deaminate the dietary protein and use it as an energy source (CYRINO et al., 2010), it is possible to turn *A. altiparanae* into a functional food that is capable of providing the essential fatty acids for human consumption through diet manipulation.

Conclusion

The replacement of animal protein with vegetable protein resulted in a saving of 15.9% on the cost of diets. The reduction in the formulation cost achieved by increasing levels of vegetable protein compensated the slight decline in biomass gain. Besides that, inclusion of vegetable protein resulted in greater fat deposition, suggesting future exploration of *A. altiparanae* as a functional food.

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