

## EFFECT OF SOYBEAN INCLUSION IN EXTRUDED RATIONS ON PERFORMANCE OF *PIAVUÇU* (*Leporinus macrocephalus* L.) JUVENILES

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### ABSTRACT

The objective of this study was to evaluate the effects of the inclusion of extruded soybean in diets for *piavuçu* (*Leporinus macrocephalus* L.) juveniles with different levels of digestible energy. A hundred fish with initial weight of  $5.06 \pm 0.61$  g were distributed into 20 aquarium (80 L), in a entirely randomized design with five treatments (0.00, 20.00, 35.00, 50.00 and 67.47% full fat soybean in the diets) and four replications. Isonitrogenous, isocalcium, isophosphorus, isoaminoacids for methionine diets with different digestible energy values (3,193; 3,326;

3,413; 3,510 and 3,621 kcal/kg) were given to fish for seventy days. The criteria evaluated were weight gain, length increment, daily feed intake, feed gain, protein efficiency ratio and survival rate. No differences ( $P>0.05$ ) were observed among treatments regarding the performance criteria. We concluded that the inclusion of full fat soybean in *piavuçu* juveniles diet is a viable option, and it may partially or totally replace soybean meal, without decreasing performance, while providing greater deposition of fat in the carcass.

KEYWORDS: alternative feedstuff; digestible energy; fish production; *Glycine max* L; nutrition.

### EFEITO DA INCLUSÃO DE SOJA INTEGRAL EM RAÇÕES EXTRUSADAS NO DESEMPENHO DE JUVENIS DE PIAVUÇU (*LeporinusMacrocephalus* L.)

### RESUMO

Com este estudo objetivou-se avaliar a inclusão de soja integral em rações extrusadas com diferentes níveis de energia digestível na dieta de juvenis de piavuçu (*Leporinus macrocephalus* L.). Foram distribuídos 100 peixes com peso inicial de  $5,06 \pm 0,41$  g, em 20 aquários (80 L), num delineamento inteiramente casualizado com cinco tratamentos (0, 20,0, 35,0, 50,0 e 67,5% de soja integral nas dietas) e quatro repetições. Dietas isoprotéicas, isocálcicas, isofosfóricas, isoaminoácidas

para metionina e com diferentes valores de energia digestível (3.193, 3.326, 3.413, 3.510 e 3.621 kcal/kg) foram fornecidas aos peixes durante setenta dias. Os seguintes critérios foram avaliados: peso final, ganho de peso, incremento em comprimento, consumo diário de ração, conversão alimentar, taxa de eficiência proteica e taxa de sobrevivência. Os tratamentos não diferiram ( $P>0,05$ ) entre si em relação aos critérios avaliados. Concluiu-se que a inclusão de soja integral nas rações para

juvenis de piavuçu é viável, podendo substituir parcial ou totalmente o farelo de soja, sem prejuízo de desempenho, embora proporcione maior deposição de gordura na carcaça.

**PALAVRAS-CHAVE:** alimento alternativo; *Glycine max* L.; energia digestível; nutrição; piscicultura.

## INTRODUCTION

The Anostomidae Characiforme is one of the most complex fish family, and the genus *Leporinus* presents around 60 described species, with broad geographic distribution and great economic importance, for both fishing and commercial exploitation. *Piavuçu* (*Leporinus macrocephalus* L.) shows great production potential for presenting fast growth, tasteful meat and rusticity (NAVARRO et al., 2007; BITTENCOURT et al., 2010). Besides, the species has omnivorous feeding habit and adapts easily to artificial diets, which makes it appropriate for intensive production.

To economically attend to the nutritive requirements of the cultivated species, aquaculture has searched for alternative sources of ingredients for feeding. FARIA et al. (2001), GALDIOLI et al. (2001), GONÇALVES et al. (2002), FURUYA et al. (2004), GENTELINI et al. (2005), SIGNOR et al. (2007) and FUNKLER et al. (2010), among others studied feeding habits, alternative foods and nutritional needs of *piavuçu*.

The expenses with feeding correspond to the greatest part of total costs, and it can surpass 50% of the expenses in the intensive productive chain (EL-SAYED, 1999), making relevant the research on use of alternative food. The possibility of reducing protein levels in the diet has been studied to decrease the costs. High levels of lipids in the diet usually increase feed conversion and the efficiency of protein fixation in the tissues. In diets with low protein:energy ration, protein is preferably used in body growth and maintenance (WINFREE & STICKNEY, 1981). The “protein saving” activity of the lipid has been discussed for some species (VAN DER MEER, 1997), however, it has not been studied for *piavuçu*.

Soybean grain has high protein and energy content and it is a good alternative of proteic food, presenting around 17 to 18% of oil and 35 to 37% of high biological value crude protein, with

favorable essential amino acids composition for feeding monogastric animals, but deficient in methionine and threonine (BELLAVÉ et al., 2002). As it contains anti-nutritional factors, which acts negatively on animal performance, in natura soybean should not be used in feeding monogastric animals without being adequately processed (MENDES et al. 2004).

According to RODRIGUES & FERNANDES (2006), fish fed extruded diet tend to improve productive performance indices. The process of extruding rations may be a way to inactivate anti-nutritional factors present in soybean and it results in an increase in digestibility of nutrients and metabolizable energy (BELLAVÉ et al., 2002), with greater productivity, costs reduction and profit increase in fish production (ROBINSON & LI, 1995). Based on this premise, we developed this study to investigate the effect of extruded diets with different inclusion levels of whole soybean and digestible energy on the performance and survival of *piavuçu* juveniles.

## MATERIAL AND METHODS

We used 100 *piavuçu* juveniles artificially reproduced from the same offspring, with mean weight and length of  $5.06g \pm 0.61g$  and  $7.30cm \pm 0.43cm$ , respectively, obtained from Universidade de Marília, SP.

The trial lasted 70 days and comprised five treatments, constituted of diets with the following inclusion levels of whole soybean: 0.00%, 20.00%, 35.00%, 50.00% and 67.5%. There were four replications per treatment. The diets were offered four times a day, at will, during the experimental period, at 10:00 a.m., 12:00 a.m., 2:00 p.m. and 4:00p.m.

The rations (Table 1) were made at the Extrusion Laboratory of Food Engineering Faculty, Universidade Estadual de Campinas, Campinas, SP.

Table 1. Percentage and nutritional composition of experimental rations with different levels of soybean inclusion

Ingredient	Level of whole soybean inclusion (%)				
	0	20	35	50	67,5
Soybean meal	50.00	35.00	24.00	13.00	0.00
Corn	46.16	41.18	36.98	33.08	28.56
Whole soybean	0.00	20.00	35.00	50.00	67.47
Fosbical	2.82	2.70	2.90	2.80	2.82
Premix	1.00	1.00	1.00	1.00	1.00
DL-Methionine	0.00	0.10	0.10	0.10	0.13
BHT	0.02	0.02	0.02	0.02	0.02
<b>Nutrient</b>					
Crude protein (%)	28.00	28.00	28.00	28.00	28.00
Ether extract (%)	2.10	5.00	7.00	9.10	11.60
Crude fiber (%)	2.70	2.70	2.70	2.70	2.70
Calcium (%)	0.80	0.80	0.80	0.80	0.80
Methionine (%)	0.75	0.75	0.75	0.75	0.75
Total phosphorus (%)	0.60	0.60	0.60	0.60	0.60
Digestible energy (kcal/kg)	3,193	3,326	3,413	3,510	3,621

Warranty vitamin and mineral levels per kg on Premix package: folic acid=250 mg; pantothenic acid=5,000 mg; vit. A=1000.000 UI; vit. B1=250 mg; vit. niacin=3750 mg; cobalt=24,999 mg; iron=11,249 mg; iodine=106 mg; manganese=3749 mg; selenium=75 mg; zinc=17,499 mg; antioxidant=0.25 g.

The rations were balanced by using the mean nutrient values of the Food Composition Table (ROSTAGNO et al., 2000) and the nutritional requirements of Nile tilapia (NRC, 1983), and formulated to fulfil different digestible energy levels (3,193.0; 3,326.0; 3,413.0; 3,510.0 and 3,621.0 kcal/kg) and be isoproteic (28% protein), isocalcium (0.8% calcium), isophosphorus (0.6% total phosphorus) and isoaminoacidic for methionine (0.75%), aiming at replacing soybean meal by whole soybean and not using any other ingredient to decrease costs.

Corn, soybean grains and soybean meal were ground separately in hammermills and then sieved (0.5 mm sieve). Homogenization was carried out in a 30-kg capacity mixer for seven minutes. The mixture was ground again and mixed one more time to improve homogeneity. The rations were prepared in laboratory corotational twin-screw extruder, equipped with online data acquisition system, programmed volumetric feeder and Watson-Marlon bomb. The processing variables were established for each ration, aiming at optimize their physical characteristics, according to SOARES JÚNIOR et al. (2004). The mixture of ingredients was placed in the volumetric feeder in the beginning of the process. The ration granules were cut by a rotating knives system attached to the exit of the extruder. The

granules were then dried in forced-air circulation greenhouse for 12 hours at 60 °C, cooled at room temperature for 20 minutes, packed in polypropylene bags and stored in refrigerator at 5 °C until use.

We used 20 fiberglass fish tanks (50 cm x 50 cm x 50 cm), with water up to 80 L, and density of five fish per unity. Each fish tank was equipped with a heater and an aerator. Each set of three (intercommunicating) tanks received a biological filter and a feeding system, and the drainage was based on vessel communication for continual and constant water renewal with approximate 0.8 L min<sup>-1</sup> flow. The fish tanks were siphoned twice a day for removing feces and any ration leftover, having the initial water volume reestablished soon after it. Water temperature was measured daily at 10:00 a.m. and 4:00 p.m. with a portable digital thermometer. Dissolved oxygen level in the water was monitored weekly by using the Winkler technique modified by the sodium azide, as described in BOYD (1984). The pH was measured weekly with a potentiometer.

We evaluated final weight, weight gain, length increase, ration intake, apparent feed conversion, protein efficiency rate and survival rate, according to the methods described by JAUNCEY & ROSS (1982), besides the proportion of crude protein and ether extract in weight gain, according to ALENCAR ARARIPE et al. (2011). Levels of body

dry matter, crude protein and ether extract of *piavuçu* fingerlings were determined in the beginning and in the end of the trial, according to AOAC (2005) official methods.

The results were evaluated with analysis of variance and the means were compared by Tukey test at 5% probability, and the levels of metabolizable energy were submitted to polynomial regression analysis, with the aid of SAS (2000).

## RESULTS AND DISCUSSION

The values of temperature in the morning and in the afternoon, dissolved oxygen and water pH were  $25.5 \pm 1.45^\circ\text{C}$ ,  $26.4 \pm 1.56^\circ\text{C}$ ,  $7.01 \pm 0.68 \text{ mg L}^{-1}$  and  $6.98 \pm 0.38$ , respectively, being within the range for tropical fish production as suggested by SIPAUBA-TAVARES (1994).

The survival rate of *piavuçu* fed with any of the diets was 100%. The percentage of whole soybean in the diet at 5% probability did not affect significantly ( $P>0.05$ ) the final weight, weight gain,

length increase, feed intake, apparent feed conversion, protein efficiency, body dry matter, body crude protein and the crude protein proportion in the weight gain; on the other hand, it affected the body ether extract and the ether extract proportion in weight gain (Table 2). The values of weight gain of *piavuçu* fingerlings overcame the results obtained by other authors who studied this species. By using extruded ration with 67.5% whole soybean, we obtained mean weight gain of  $0.93 \text{ g}\cdot\text{day}^{-1}$  or 18.35%, while NAGAE et al. (2002) observed maximum value of  $0.25 \text{ g}\cdot\text{day}^{-1}$  in fingerlings with initial weight of 1.38 during 60 days, by using cold pelleted rations constituted of millet as a replacement for corn. Although these diets had the same protein level, the energy level was inferior than the one used in this study. On the other hand, GONÇALVES et al. (2002) used *piavuçu* fingerlings with mean initial weight of 8.51 g and cold pelleted rations with 30% protein, 3000 kcal and different inclusion levels of canola meal to the ration and verified maximum weight gain of 8.68% by day.

Table 2. Mean values of performance and body dry matter, crude protein and ether extract of *piavuçu* juveniles (*Leporinus macrocephalus* L.) in function of the diets with different inclusion levels of whole soybean during 70 days

Performance	Level of whole soybean inclusion (%)					CV	P Value
	0	20	35	50	67.5		
Final weight (g)	52.3±7.8	59.4±6.5	60.6±17.3	62.4±12.3	70.1±13.9	23.1	0.54
Weight gain (g)	47.3±7.8	54.3±6.5	55.6±17.3	57.4±12.3	65.0±13.9	25.3	0.54
Weight gain (g)	934.6±153.9	1073.8±128.6	1098.5±342.2	1134.0±242.3	1284.8±276.1	25.3	0.54
Length increase (cm)	8.7±0.7	8.7±0.2	8.8±1.8	9.54±0.8	10.2±1.2	14.1	0.40
Feed intake (g)	56.6±6.8	58.34.5	63.0±4.0	66.32±5.42	66.4±9.1	10.2	0.17
Apparent feed conversion	1.1±0.2	1.1±0.1	1.0±0.5	1.02±0.20	1.0±0.1	14.3	0.78
Protein efficiency	3.0±0.4	3.2±0.2	3.4±0.9	3.18±0.52	3.3±0.4	7.9	0.78
Dry matter (g.100g <sup>-1</sup> )	16.94±1.8	17.11±1.5	17.86±0.7	16.79±1.9	17.80±1.2	8.1	0.77
Body crude protein (g.100g <sup>-1</sup> )	9.51±1.1	8.90±0.6	9.53±0.6	8.63±0.9	8.63±0.5	8.9	0.31
Body ether extract (g.100g <sup>-1</sup> )	5.03 <sup>B</sup> ±0.47	6.37 <sup>AB</sup> ±0.6	6.60 <sup>AB</sup> ±0.8	6.74 <sup>AB</sup> ±0.9	7.59 <sup>A</sup> ±1.4	18.0	0.02
Crude protein in weight gain	9.99±1.2	9.25±0.7	10.0±0.9	9.0±1.0	8.9±0.6	10.0	0.28
Ether extract in weight gain	5.51 <sup>B</sup> ±0.5	6.91 <sup>AB</sup> ±0.7	7.23 <sup>AB</sup> ±1.1	7.38 <sup>AB</sup> ±1.0	8.16 <sup>A</sup> ±1.5	18.1	0.03

\*Means with different letters in the same row differ statistically between each other at 5% error probability by Tukey test.

The greatest values in weight gain obtained in this study are probably due to the processing of the ration, because the extrusion technology allows the obtainment of ration granules with higher stability in water, higher physical integrity, and with adequate form and size to the fish size, besides inactivating inhibitors and starch gelatinization, improving the product digestibility (VIOLA et al.; 1983; KIANG, 1999; SOARES JÚNIOR et al.,

2004). Furthermore, the use of whole soybean increased the energy level of the rations.

Decreasing the time between grinding the soybean grain and the ration extrusion is important to make feasible the use of raw whole soybean in extruded rations. Therefore, undesirable chemical reactions should be avoided, such as lipases and lipoxygenases activity on triacylglycerides released from the vegetable cells during the interval between

these operations.

The fish reached final length between 15.99 and 17.55 cm (Table 2). These values are higher than the ones observed by NAGAE et al. (2002), who found variation ranging from 9.82 and 10.39 cm by evaluating millet inclusion in *piavuçu* rations, as well as the ones verified by NAGAE et al. (2001), with variation from 9.07 to 9.61 cm for total length, by studying triticale inclusion in fingerlings rations with mean initial weight of  $1.70 \pm 0.03$  cm for 60 days. Comparing studies carried out with other species, ZEITLER et al. (1984) observed higher growth rate of the common carp (*Cyprinus carpio* L.) by incrementing the ration with digestible energy, regardless of the protein level used. VIOLA et al. (1983) reported that the use of supplement oil in the ration of Nile tilapia (*Oreochromis niloticus*) did not affect significantly growth gain and feed efficiency, contrary to fish such as the common carp (*Cyprinus carpio* L.), the rainbow trout (*Oncorhynchus mykiss*) and the American catfish (*Ictalurus punctatus*), which use the supplement oil efficiently.

Feed intake of the evaluated rations allowed us to state that any of them was rejected. In control treatment the feed intake was, on average, 16% lower than in the treatment with 67.5 whole soybean (Table 2). In an experiment with *piavuçu* carried out by GONÇALVES et al. (2002), the reported values were lower and ranged between 41.10 and 43.5 g, while FURUYA et al. (2004), using whole soybean meal as a replacement for soybean meal in diets for Nile tilapia juveniles (mean initial weight of  $1.58 \pm 0.06$  g) during 45 days, verified intake values ranging from 13.14 to 16.23 g.

Feed conversion ranged from 1.01 (with 67.5% whole soybean) and 1.14 (control treatment), that is, almost 13% (Table 2). FARIA et al. (2001) studied diets with partial or total replacement of fish meal by soybean meal for *piavuçu* fingerlings with mean initial weight of 0.52 g during 62 days and observed variation between 1.11 and 1.32% in feed conversion. As for the ration formulated with soybean meal and without fish meal, the authors observed feed conversion values similar to the ones verified in this study.

HANLEY (1991) fed young tilapia with rations with 5%, 9% and 12% lipids and observed that the increase in lipid content in ration did not affect significantly growth rate, feed conversion rate and protein efficiency, agreeing with the results found in this study with *piavuçu*, where lipid levels varied between 2.1 and 11.6% and the difference among treatments effects was not significant.

The protein efficiency rate in the rations used in this trial varied between 3.00 and 3.36, and these values were higher than those found by

GALDIOLI et al. (2001), NAGAE et al. (2001), FARIA et al. (2001) and FURUYA et al. (2004), and within the range verified by GONÇALVES et al. (2002). VAN DER MEER et al. (1997) also verified the effect of lipid level in the diet on the protein use in tambaqui (*Colossoma macropomum*) juveniles, verifying that growth and protein use efficiency increased with the lipid level in the ration.

The mean dry matter of *piavuçu* fingerlings determined in the beginning of the trial was 8.04 g.100g<sup>-1</sup>, body crude protein was 5.12 g.100g<sup>-1</sup> and body ether extract was 0.65 g.100g<sup>-1</sup>. After ten weeks, duration of the trial, these values increased between 108-122%, 69-86%, and 674-1068%, respectively. The diets did not affect dry matter and body crude protein content, but they affected body ether extract content, which varied 51% between the diet with zero and with 67.5% whole soybean (Table 2). The treatment without whole soybean differed from the one with 67.5%, while the other treatments did not differ among each other. Crude protein in weight gain varied only 12% among treatments, while ether extract in weight gain was affected by the diet, varying 48% (Table 2). The treatment without whole soybean differed from the one with 67.5%, while the other treatments did not differ among each other. HANLEY (1991) observed, in an experiment with tilapia, that the increase in lipids level in the ration did not alter significantly moisture and protein content of the fish, but it increased significantly lipids content in the carcass and viscera. The percentage of fat in the carcass and the level of fat deposition in weight gain increased ( $p < 0.001$ ) linearly in function of the increase in the energy level of the diet for *piavuçu* in post-larval phase (NAVARRO, 2007). VIOLA et al. (1988) also observed that the tilapia that received ration with supplement oil, compared to the ones that received basal ration, presented 40% more fat deposition in the carcass, which confirms the values found in this study.

## CONCLUSIONS

We concluded that the inclusion of whole soybean to the rations for *piavuçu* juveniles is feasible, and it can replace partially or totally soybean meal, without damaging fish performance, even with higher energy levels, although it produces greater fat deposition in the carcass.

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