REPLACEMENT OF ANIMAL PROTEIN SOURCES BY SOY PROTEIN CONCENTRATE FOR JUVENILE NILE TILAPIA

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ABSTRACT

Soy protein concentrate (SPC) was evaluated as a dietary replacement of animal protein sources (fish meal and poultry by-product meal) (APS) for juvenile Nile tilapia (*Oreochromis niloticus*). Apparent digestibility coefficients (ADC) of crude protein (CP) and dry matter (DM) were evaluated by feeding tilapia an experimental diet composed of 69.5% reference diet, 30% SPC, and 0.5% chromic oxide as inert marker. Apparent digestibility coefficients were 96.57% for CP and 76.84% for DM. In a feeding trial, increasing levels of dietary SPC (0, 33, 67, and 100%) replaced APS and were fed to tilapia juveniles (10.0 ± 0.18 g) for 60 days. Daily weight gain, specific growth rate, protein retention, feed conversion, body composition and liver histology were not significantly (P>0.05) affected by protein replacement. A second feeding trial compared tilapia's growth performance when SPC was supplemented with methionine and threonine (100% SPC+aa, 100% SPC, and 0% SPC) as well as CP and DM digestibility. The amino acid supplementation of SPC significantly increased tilapia daily weight gain. Diets containing 100%SPC and 100% SPC+aa promoted higher protein ADC values than diet containing only APS. However, DM ADC values were significantly higher in fish fed 0% SPC when compared to 100% SPC. Therefore, SPC can replace poultry by-product meal and fish meal in diets for Nile tilapia without compromising growth performance, protein retention, body composition, liver histology, and protein digestibility. However, SPC supplementation with limiting amino acids, such as methionine and threonine, is advisable since it further increases weight gain and protein digestibility.

Keywords: nutrition; aquaculture, fishmeal; freshwater fish; *Oreochromis niloticus*

SUBSTITUIÇÃO DE FONTES DE PROTEÍNA ANIMAL PELO CONCENTRADO PROTEICO DE SOJA PARA JUVENIS DE TILÁPIA DO NILO

RESUMO

Concentrado proteico de soja (CPS) foi avaliado como substituto dietético das farinhas de peixe e vísceras de aves (FP e FVA) para juvenis de tilápia do Nilo (*Oreochromis niloticus*). Os coeficientes de digestibilidade aparente (CDA) da proteína bruta (PB) e matéria seca (MS) foram avaliados com o fornecimento de dieta experimental composta de 69,5% da dieta referência, 30% CPS e 0,5% de óxido de cromo como marcador inerte. O CDA foi de 96,57% para PB e 76,84% para MS. Em ensaio de alimentação, dietas com níveis crescentes de CPS (0, 33, 67 e 100%) foram oferecidas para juvenis de tilápia (10,0 ± 0,18 g) por 60 dias. Ganho de peso diário, taxa de crescimento específico, retenção proteica, conversão alimentar, composição corporal e histologia hepática não foram afetados (P>0,05) pela substituição. Um segundo ensaio de alimentação comparou crescimento e digestibilidade quando CPS foi suplementado com metionina e treonina (100% CPS+aa, 100% CPS, e 0% CPS). A suplementação resultou em maior ganho de peso diário dos peixes. As dietas 100% e 100% CPS+aa promoveram maiores CDA para proteína que a dieta 0% CPS. No entanto, o CDA para MS foi significativamente maior nos peixes alimentados com 0% CPS quando comparado com 100% CPS. CPS pode substituir FP e FVA em dietas de tilápia do Nilo sem comprometer o desempenho do crescimento, retenção proteica, composição corporal, histologia hepática e digestibilidade proteica. A suplementação de CPS com aminoácidos limitantes, como metionina e treonina, é aconselhável, pois aumenta o ganho de peso e digestibilidade proteica.

Palavras-chave: nutrição; aquicultura; farinha de peixe; peixe de água doce; *Oreochromis niloticus*

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INTRODUCTION

Nile tilapia is an omnivorous species suited both for extensive and intensive farming. Nile tilapia's intestines are around six times its total length which provides a large surface area for digestion and nutrient absorption from the diet. Therefore, Nile tilapia efficiently utilizes protein and energy from plant sources (GOMINHO-ROSA *et al*., 2014) as well as from animal sources.

Although fishmeal is a valuable protein source in diets for aquaculture, its inclusion in Nile tilapia commercial diets is usually very low due to cost restrains. Other animal protein sources, readily available in Brazil, can be used in tilapia diets, such as poultry by-product meal; although, its higher fat content and variable composition (FERNANDES, 2011) can restrict its inclusion in tilapia feeds.

Soybean meal is traditionally used as a protein source in tilapia commercial feeds, with 20 to 30% total protein included (TSUKAMOTO and TAKAHASHI, 1992). However, anti-nutritional factors, low palatability, as well as deficiency in certain amino acids and essential fatty acids can represent some constraints (RIBEIRO, 2012). On the other hand, soybean protein concentrate (SPC) has higher crude protein content, equivalent to approximately 65%, similar to that observed in fish meal, and low levels of anti-nutritional factors, despite still presenting deficiency in some essential amino acids such as methionine (KAUSHIK *et al*., 1995). Brazil is the second largest world producer of soybeans (BRASIL, 2012). Soybean protein concentrate is obtained by removing the oil and the protein fraction, which is not soluble in water, by washes with an alcohol solution. This washing removes and/or denatures soybean ant nutritional factors, in addition to the elimination of residual lipids (CARVALHO, 2011).

Soy protein concentrate has been tested on some species of fish and shrimp such as rainbow trout, *Oncorhyncus mykiss* (MAMBRINI *et al*., 1999), Atlantic salmon, *Salmo salar* (STOREBAKKEN *et al*., 1998), tiger shrimp, *Penaeus monodon* (PARIPATANANONT *et al*., 2001), and Pacific white shrimp, *Litopenaeus vannamei* (SÁ *et al*., 2013) and indicated a good potential to replace animal source protein. Additionally, methionine supplemented SPC promoted adequate growth for rainbow trout (KAUSHIK *et al*., 1995) and Nile tilapia less than 2 g (ZHAO *et al*., 2010), respectively, when totally replacing fish meal protein.

The present study aimed to evaluate increasing levels of animal protein (fish meal and poultry by-product meal) replacement with SPC in juvenile Nile tilapia's diets. We also evaluated tilapia's protein and dry matter digestibility of SPC, and examined liver histology for any hepatic dysfunction.

MATERIAL AND METHODS

Soy protein concentrate digestibility trial

Nile tilapia's protein and dry matter apparent digestibility coefficients of SPC were evaluated in a completely randomized design feeding trial using two dietary treatments in triplicate: 1) basal diet, consisting of semi purified ingredients, and 2) test diet containing 69.5% basal diet and 30% SPC. Basal diet contained 0.5% chromium oxide, as an inert marker, to calculate apparent digestibility coefficients (Table 1).

Groups of 13 tilapia (185.97 ± 14.32 g; mean ± standard deviation), GIFT strain, purchased from a commercial supplier (Piscicultura Acquasul Cay, SC), were stocked into 200-L cylinder-conical tanks, totaling approximately 2,340 g biomass per tank. Fish were acclimated to experimental conditions for a week. Tanks, coupled to a closed recirculating water system with constant aeration and temperature control (27.0 ± 1 °C), were equipped with tubes for feces collection. Water quality variables were monitored daily and were within normal range for tilapia (POPMA and LOVSHIN, 1994), as follows (mean ± standard deviation): dissolved oxygen = 4.19 ± 0.14 mg L^{-1}; temperature = 27.2 ± 0.16 °C; pH = 6.23 ± 0.06. Photoperiod was kept at 12 h and water exchange rate at 1.5 L min^{-1}.

For experimental diet preparation, dry ingredients were homogenized in a mixer oil and water was added subsequently. The resulting mixture was extruded into a 4 mm die and dried at 55 °C in a forced air oven. Fish were fed twice a day (10:00 h and 16:00 h) to satiation. Before feces collection, approximately one hour after last

feeding, thank internal walls were cleaned and approximately 70% of the water was replaced to avoid contamination of feces with diets. Fecal collection tubes were placed at the bottom of the tanks, where feces settled for later collection, according to the methodology proposed by KITAGIMA (2009). Feces collection was performed three times a day at 6-h intervals (23:00 h, 05:00 h and 09:00 h). After collection, feces were centrifuged (2.296 x *g* during 5 min), dried at 60 °C, grinded and stored at -20 °C for further analysis.

Table 1. Formulation and proximate composition of the experimental diets used in the digestibility trial (dry matter basis).

Ingredient (%)	Diets	
	Basal	Test
Soy protein concentrate[1]	0.0	30.0
Casein[2]	28.3	19.8
Gelatin[3]	8.8	6.1
Starch[4]	48.5	34.0
Celullose[5]	4.0	2.8
Fish oil[6]	2.0	1.4
Soybean oil[7]	2.9	2.0
Phosphate dicalcium[8]	2.0	1.4
Micro mineral and vitamin premix[9]	1.0	0.7
Macro mineral premix[10]	2.0	1.4
Choline chloride[11]	0.1	0.04
Chromium oxide III [12]	0.5	0.4

[1]IMCOPA - Importação, Exportação e Indústria de Óleos S.A. (Araucaria, PR). [2,3,4,5,11]RHOSTER - Indústria e Comércio Ltda (Araçoiaba da Serra, SP). [6]Delaware (Porto Alegre, RS).[7]BUNGE (Gaspar, SC). [8]TORTUGA(São Paulo, SP). [9]VACCINAR (Pinhais,PR). Composition (kg^{-1} product): 1,200 mg folic acid; 10,000 mg pantothenic acid; 200 mg biotin, 100,000 mg choline; 20,000 mg niacin; 2,400,000 IU vitamin (vit.) A; 4,000 mg vit. B1; 8,000 mg vit. B12; 4,000 mg vit. B2; 3,500 mg vit. B6; 60,000 mg vit. C; 600,000 IU vit. D3; 30,000 IU vit. E; 3,000 mg K; 80 mg cobalt; 3,500 mg copper; 20,000 mg iron; 160 mg iodine; 10,000 mg manganese; 100 mg selenium; 24,000 mg zinc; 25,000 mg Inositol. [10]VACCINAR (Pinhais, PR). Composition (kg^{-1} product): 45.40% phosphate dicalcium; 29.70% potassium chloride; 17.40% sodium chloride; 7.50% magnesium sulfate. [12]VETEC Química Fina Ltda (Rio de Janeiro, RJ).

The apparent digestibility coefficients (ADC) of dry matter and protein were calculated following the equation proposed by CHO and SLINGER (1979):

$$ADC = 100 - [100 (Cr_2O_3d/Cr_2O_3f \times Fn/Dn)],$$

where: ADC (%): apparent digestibility coefficient; Cr_2O_3d (%): chromium oxide in the diet; Cr_2O_3f (%): chromium oxide in feces; Fn (%): nutrient in the feces-Dn (%): nutrient in the diet.

Protein replacement growth trial

The animals were handled in accordance with the Ethics Committee on Animal Use the Federal University of Santa Catarina, protocol number 88/CEUA/PRPE/2012 (CEUA, UFSC). Nile tilapia from the GIFT strain were purchased from the same commercial supplier as described earlier and kept in a closed recirculating water system with mechanical and biological filtration, aeration and temperature control. Water quality variables followed the same pattern as the previous trial, as follows (mean ± standard deviation): dissolved oxygen = 6.30 ± 0.46 mg L^{-1}; temperature = 28.36 ± 0.28 °C; pH = 7.20 ± 0.19. Photoperiod was kept at 12 h and water exchange rate at 1 L min^{-1}.

Fish were acclimated to experimental conditions for a week, when they were fed a

commercial diet containing 35% crude protein. Five diets with increasing levels of SPC replacing animal protein (0%, 33%, 67%, 100% and 100% SPC + amino acid supplementation) were randomly allocated in triplicate to groups of 25 juvenile Nile tilapia (initial weight (mean ± standard deviation = 10.00 ± 0.18 g), housed in 120-L tanks. The experimental diets (Table 2) were formulated based on the nutritional requirements for Nile tilapia (NRC, 2011). Methionine and threonine were supplemented in one of the 100% replacement diets since their levels were below Nile tilapia's requirements (Table 3). Diets were offered at 3% of body weight, twice daily (9:00 h and 16:00 h) for 60 days. Diets were prepared as detailed in the digestibility trial, except for pellet size, which was 2 mm.

Samples of 30 fish from the initial stock and three fish per tank (nine fish/dietary treatment) at the end of the experiment were collected to determine whole body composition for calculating apparent net protein utilization. Fish were euthanized by an overdose (1 ml L^{-1}) of clove oil (Eugenol®, Biodynamic Chemicals & Pharmaceuticals Ltd., Ibiporã, PR), and stored (-20 °C) for later analyzes.

Table 2. Formulation and proximate composition of the experimental diets used in Nile tilapia growth trial (dry matter basis).

Ingredient (%)	**Protein replacement (%)**				
	0	**33**	**67**	**100**	**100% SPC+aa[1]**
Poultry by-product meal[2]	42.60	29.36	14.55	0.00	0.00
Fish meal[3]	4.00	4.00	4.00	0.00	0.00
Soy protein concentrate[4]	0.00	4.00	29.82	49.30	49.10
Methionine[5]	0.00	0.00	0.00	0.00	0.15
Threonine[6]	0.00	0.00	0.00	0.00	0.15
Corn[7]	42.58	39.82	36.80	36.00	36.00
Cellulose[8]	6.50	7.00	7.20	4.99	4.89
Soybean oil[9]	0.00	1.50	3.31	5.39	5.39
Premix macro mineral[10]	2.00	2.00	2.00	2.00	2.00
Phosphate dicalcium[11]	1.10	1.10	1.10	1.10	1.10
Micro mineral and vitamin premix[12]	1.00	1.00	1.00	1.00	1.00
Butylated hydroxytoluene[13]	0.22	0.22	0.22	0.22	0.22
Proximate composition (% dry matter) [14]					
Digestible protein	33.02	32.72	32.20	33.72	33.30
Crude fat	9.90	9.38	8.42	8.83	8.38
Ash	10.18	9.50	8.47	7.73	7.33
Acid detergent fiber	10.92	6.76	9.02	9.79	10.40
Gross energy (kcal kg^{-1})[15]	4275	4442	4332	4377	4337

[1]100% soy protein concentrate + methionine and threonine supplementation. [2]Kabsa S.A. (Porto Alegre, RS). [3]TECTRON Nutrição Animal (Toledo, PR). [4]IMCOPA - Importação, Exportação e Indústria de Óleos S.A. (Araucaria, PR, Brazil). [5]MetAMINO®: DL-Methionine feed grade 99%. [6]ThreAMINO®: L-Threonine feed grade 98.5%, Evonik Industries (Cascavel, PR). [7]Nicoluzzi Rações Ltda (Penha, SC). [8]RHOSTER - Industry and commerce Ltda. (Araçoiaba da Serra, SP). [9]BUNGE (Gaspar, SC). [10,12]VACCINAR (Pinhais, PR). [10]Composition of product Kg^{-1}: 1,200 mg folic acid; 10,000 mg pantothenic acid; 200 mg biotin; 100,000 mg choline; 20.000 mg niacin; 2,400,000 IU vitamin (vit.) A; 4,000 mg vit. B1; 8,000 mg vit.B12; 4,000 mg vit. B2; 3,500 mg vit. B6; 60,000 mg vit. C; 600,000 IU vit. D3; 30,000 IU vit. E; 3,000 mg k; 80 mg cobalt; 3,500 mg copper; 20,000 mg iron; 160 mg iodine; 10,000 mg manganese; 100 mg selenium; 24,000 mg zinc; 25,000 mg Inositol. [12]Composition of the product Kg^{-1}: 45.40% fosfato bicálcico; 29.70% potassium chloride; 17.40% sodium chloride; 7.50%magnesium sulfate. [11]TORTUGA (São Paulo, SP). [13]Labsynth® (Diadema, SP). [14]According AOAC (1999). [15]According NRC(2011).

Table 3. Amino acid profile of experimental diets used in the growth trial and amino acid requirements of Nile tilapia (*Oreochromis niloticus*).

Amino acids (%)	Nile tilapia nutritional requirements[1]	Protein replacement (%)			
		0	33	67	100
Arginine	1.2	2.21	2.12	2.03	1.95
Histidine	1.0	1.01	1.07	1.04	1.01
Isoleucine	1.0	1.27	1.32	1.38	1.44
Leucine	1.0	2.36	2.46	2.59	2.73
Methionine+Cystine	0.7	1.35	1.17	0.98	0.59
Threonine	1.1	1.19	1.12	1.05	0.97
Valine	1.5	1.42	1.42	1.43	1.44
Tryptophan	0.3	0.33	0.35	0.36	0.43
Phenylalanine+Tyrosine	1.6	2.07	2.08	2.09	2.12
Lysine	1.6	2.48	2.35	2.21	2.07

[1]*Amino acid profile of experimental diets was calculated based on ingredient amino acid contents according to NRC (2011).*

The variables of the dose-response trial were calculated using the following equations:

- Weight gain: WG (g) = final weight – initial weight;
- Feed conversion: FC = feed consumption (dry weight)/weight gain;
- Feed efficiency: FE = weight gain/feed consumption (dry weight);
- Specific Growth Rate: SGR (%) = {(ln final weight – ln initial weight) /days on trial} × 100;
- Apparent Net Protein Utilization: ANPU (%) = (net increase in whole body protein/amount of protein consumed) ×100.

Fish weight gain in each experimental unit was monitored through individual biweekly weighing. Before weighing, fish were fasted for 24 h, and fish were kept under anesthesia (Eugenol®) during the whole procedure.

At the end of the growth trial, fish were kept for 60 days in the same experimental set up as the growth trial for fecal collection estimate experimental diet digestibility. Fish were fed the same diet as in the growth trial, except that they contained 0.5% of chromic oxide as an inert marker. Fish were fed twice daily (10:00 h and 16:00 h). To avoid feces contamination, approximately one hour before feeding, tanks were siphoned to remove existing feces and about 70% of the water in each tank was renewed. Fish were carefully fed to apparent satiation, to avoid any leftover feed in the tanks. One hour after feeding, feces were collected in a beaker by siphoning. After collection, feces were centrifuged, dried at 60 °C, grinded and stored (-20 °C) for further analysis. Protein and dry matter apparent digestibility coefficients were calculated following the equation proposed by CHO and SLINGER (1979).

Histology

At the end of the growth trial, three fish from each experimental unit were euthanized by an overdose of clover oil, Eugenol® (1 ml L^{-1}), and dissected to remove the liver. Liver samples were fixed in Bouin solution for 24 h, followed by a wash in 70% ethanol for 1 h. Paraffin inclusion of tissue followed routine methodology, at 58 °C, using xylene as an intermediate fluid (CARGNIN-FERREIRA and SARASQUETE, 2008). The resulting blocks were cut in a microtome (Leica RM 2025) at 5 micrometers. These cuts were extended and collected at 52 °C bath and placed on slides. Tissue sections were deparaffinized, hydrated, and colored with Giemsa diluted in distilled water. Slides were analyzed and photographed with a photomicroscope (Leica ICC50 HD, São Paulo, SP, Brazil), using the LAS EZ software, with an increase of 100 times.

Diets, feces and body composition analyses

Proximate analyzes of diet, feces, and body composition for all feeding trials was performed

according to standard procedures (AOAC, 1999). Chromic oxide concentration of diets and feces were determined by the diphenyl carbazide spectrophotometric method, according to the methodology proposed by BREMER *et al*. (2005).

Statistical analyses

To determine the best concentration of SPC for growth, nutrient retention, and feed conversion data from all dietary treatments (except 100% SPC+aa) were subjected to regression analysis. The apparent digestibility data from experimental diets were analyzed by analysis of variance (ANOVA), followed by Tukey test (ZAR, 2009). ANOVA was used to compare dietary treatments 0%, 100% and 100% SPC+aa. All analyzes were performed with the software Statistica 7.0 (STATSOFT Inc., 2004), adopting a significance level of 5%.

RESULTS

Protein and dry matter apparent digestibility coefficients of SPC for Nile tilapia are presented in Table 4.

Table 4. Apparent digestibility coefficients (ADC) of soybean protein concentrate for Nile tilapia. Values represent means and respective standard errors of means (SEM).

Variable	ADC (%)
Crude protein	96.57 ± 0.18
Dry matter	76.84 ± 2.47

In growth assays, weight gain, specific growth rate, feed conversion, daily weight gain and protein retention were not affected (P>0.05) by increasing levels of replacement of dietary animal protein by SPC (Table 5, Figure 1).

Table 5. Growth and feed efficiency variables of Nile tilapia juveniles fed with diets containing increasing levels of animal protein replacement (poultry by-product meal and fish meal) with soybean protein concentrate for 60 days. Values represent means and respective standard errors of means (SEM).[1,2]

Variable	Protein Replacement (%)				
	0	33	67	100	*P* value
Final weight (g)	53.28 ± 1.55	57.02 ± 0.72	57.96 ± 0.66	54.61 ± 0.81	0.0142
Specific growth rate (%)	2.76 ± 0.08	2.91 ± 0.03	2.90 ± 0.08	2.84 ± 0.07	0.7568
Feed conversion	1.28 ± 0.046	1.19 ± 0.049	1.47 ± 0.027	1.30 ± 0.053	0.4611
Feed efficiency	3.42 ± 0.40	3.42 ± 0.16	2.89 ± 0.03	3.30 ± 0.11	0.1790
Feed consumption (g fish^{-1})	50.87 ± 3.80	55.15 ± 3.35	66.02 ± 0.31	54.17 ± 1.03	0.0030

[1]*Initial weight = 10.00 ± 0.18 g.* [2]*Variables found for 100 SPC+aa fed fish were not included in the regression analysis. Their values are: final weight: 59.78 ± 2.91 g; specific growth rate: 2.94 ± 0.047%; feed conversion: 1.59 ± 0.066; feed efficiency: 3.31 ± 0.087; feed consumption, 59.92 ± 4.15 g fish^{-1}.*

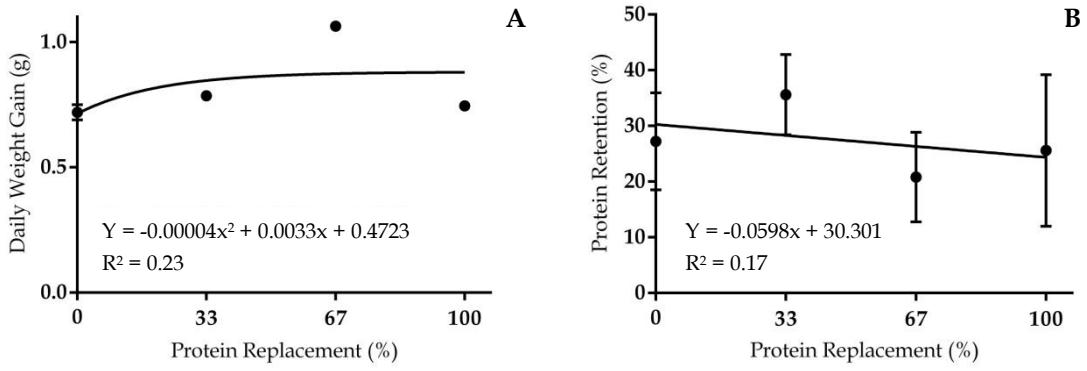

Figure 1. Polynomial regression (P>0.05) of daily weight gain (A) and protein retention (B) of Nile tilapia juveniles, when fed with diets increasing levels of animal protein replacement (poultry by-product meal and fish meal) with soybean protein concentrate for 60 days. Values represent means and respective standard errors of means (SEM) for protein retention. Standard error of means for 0%, 33%, 67%, and 100% daily weight gain (A) averages were 0.0270, 0.0107, 0.0121 and 0.0164 g, respectively.

Similarly, there were no differences on specific growth rate, feed conversion or protein retention when Nile tilapia juveniles were fed diets containing only animal protein sources (poultry by-product meal and fish meal), SPC or SPC supplemented with methionine and threonine. However, daily weight gain was significantly higher (P<0.05) in fish fed 100% soybean protein concentrate supplemented with amino acids when compared to those fed the non-supplemented diet (Figure 2).

Fish body composition was not affected by increasing levels of protein replacement with SPC or by supplementing SPC with amino acids (P>0.05) (Tables 6 and 7).

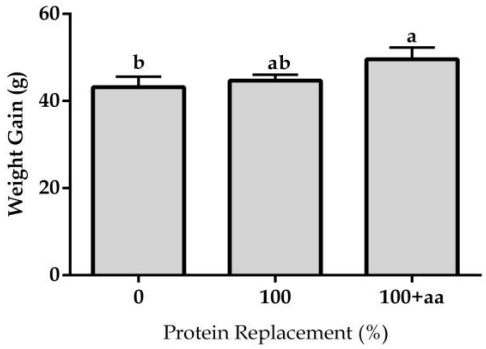

Figure 2. Daily weight gain of juvenile Nile tilapia fed diets containing no soybean meal concentrate (SPC), 100%SPC, and 100%SPC plus supplemented amino acids (see Table 1). [a,b] Bars with the same letters are not significantly different by Tukey test (P>0.05).

Table 6. Body composition of juvenile Nile tilapia (wet basis) when fed diets containing increasing levels of soy protein concentrate in replacement of animal protein. Values are means and respective standard errors of means (SEM).

Body composition (%)	Protein replacement (%)					*P* values
	0	33	67	100	100+aa[1]	
Crude protein	11.26 ± 3.56	13.73 ± 2.72	10.51 ± 3.73	11.30 ± 6.13	15.11 ± 1.70	0.48162
Lipid	8.06 ± 0.16	8.39 ± 0.49	6.32 ± 1.38	8.46 ± 0.81	7.01 ± 0.83	0.08790
Ash	3.66 ± 0.07	4.29 ± 0.59	3.46 ± 0.58	2.59 ± 0.39	2.78 ± 0.34	0.42633
Dry matter	26.36 ± 0.51	27.50 ± 1.40	24.63 ± 1.42	25.77 ± 0.71	25.53 ± 0.50	0.38613

[1]*aa = supplementation with methionine and threonine.*

Table 7. Body composition of juvenile Nile tilapia (wet basis) when fed diets containing no soybean meal concentrate (SPC), 100% SPC, and 100% SPC plus supplemented amino acids. Values are means and respective standard errors of means (SEM).

Body composition (%)	Protein replacement (%)			*P* values
	0	100	100+aa[1]	
Crude protein	11.26 ± 3.56	11.30 ± 6.13	15.11 ± 1.70	0.48162
Lipid	8.06 ± 0.16	8.46 ± 0.81	7.01 ± 0.83	0.08790
Ash	3.66 ± 0.07	2.59 ± 0.39	2.78 ± 0.34	0.42633
Dry matter	26.36 ± 0.51	25.77 ± 0.71	25.53 ± 0.50	0.38613

[1]*aa = supplementation with methionine and threonine.*

In the digestibility assay, protein and dry matter ADC were not affected (P>0.05) by increasing levels of replacement of dietary animal protein by soybean protein concentrate (Table 8). Protein ADC was high in all diets, whereas the digestibility of dry matter showed lower values (Table 8). Diets containing SPC as the sole protein source or SPC plus amino acid supplementation promoted higher protein digestibility than diets without SPC (P<0.05), but no difference were registered between them (P>0.05) (Table 9). Dry matter ADC was significantly higher (P<0.05) in fish fed diet without SPC when compared to fish fed diet

containing 100% SPC. Dry matter ADC for fish fed 100% SPC+aa presented intermediary values (*P*>0.05) (Table 9).

Hepatic histology showed normal structures regardless of protein replacement level or amino acid supplementation (Figure 3).

Table 8. Apparent coefficient digestibility (ADC; %) of protein and dry matter of diets containing increasing levels of soy protein concentrate replacing animal protein. Values are means and respective standard errors of means (SEM).

ADC (%)	Protein Replacement (%)				*P* values
	0	33	67	100	
Protein	86.30 ± 1.13	86.79 ± 1.69	91.56 ± 0.21	89.47 ± 2.28	0.2447
Dry Matter	70.20 ± 1.31	66.56 ± 3.70	70.99 ± 1.01	64.16 ± 2.09	0.4452

Table 9. Apparent coefficient digestibility (ADC; %) of protein and dry matter of diets containing no soybean meal concentrate (SPC), 100%SPC, and 100%SPC plus supplemented amino acids. Values are means and respective standard errors of means (SEM).

ADC (%)	Protein Replacement (%)		
	0	100	100+aa
Protein	86.30 ± 1.13^{b}	89.47 ± 2.28^{a}	92.38 ± 0.58^{a}
Dry Matter	70.20 ± 1.31^{a}	64.16 ± 2.09^{b}	67.93 ± 0.84^{ab}

^{a,b} Averages followed by the same letters in rows are not significantly different by Tukey test (P>0.05).

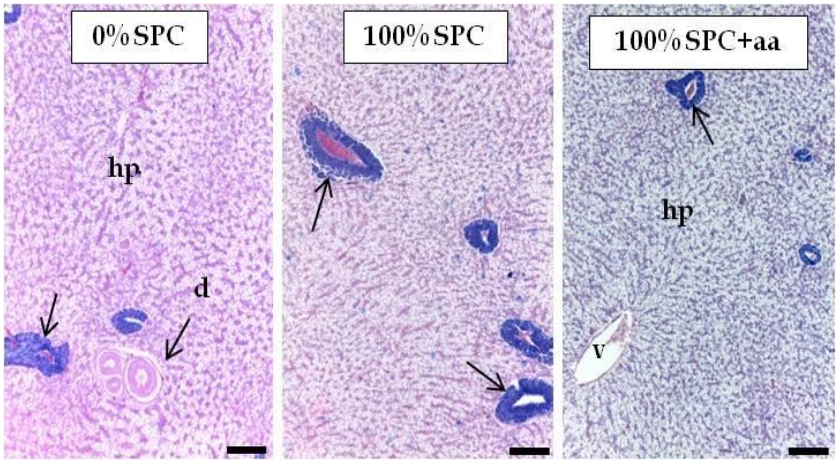

Figure 3. Histological sections showing the normal liver architecture of juvenile Nile tilapia in the extreme levels of replacement of dietary animal protein with soy protein concentrate. Note typical arrangement of the hepatocyte plates, with normal sinusoids, central veins (v) and ducts (d). Diffused pancreatic tissue (arrow) appears disperse in the hepatic parenchyma (hp). Bar: 200 µm; staining: Giemsa.

DISCUSSION

Our findings showed that SPC is a good protein source for juvenile Nile tilapia since growth and protein retention were not significantly affected when SPC replaced completely animal protein sources (poultry by-product meal and fish meal). Protein apparent digestibility coefficient (ADC) of SPC to Nile tilapia was high and similar to that reported to hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus)*, which was 98.2% (DONG *et al*., 2010). Dry matter ADC values were

lower but still higher than that reported for hybrid tilapia (67.5%) in the same study. Additionally, body composition and liver histology did not show deleterious effects of replacing animal protein with SPC.

However, we also registered that methionine and threonine supplementation of 100% SPC diet promoted an 11% increase in tilapia weight gain and showed significant effect on protein ADC. Therefore, despite being a good alternative source for replacing animal protein, SPC supplementation with the limiting amino acids is mandatory. Indeed, methionine is usually the first limiting amino acid in fish diets with high levels of soybean products (ESPE *et al*., 2008). Methionine supplementation of SPC was also found to promote significantly higher weight gain for smaller Nile tilapia (2.0 g), when compared to diet containing 100% SPC without amino acid supplementation (ZHAO *et al*., 2010). Only methionine was supplemented in that study whereas methionine and threonine were limiting in our study and both were supplemented to meet Nile tilapia's requirement (NRC, 2011). Indeed, protein digestibility increased significantly for Nile tilapia when SPC totally replaced fish meal alone or when supplemented with methionine and threonine. However, dietary amino acid supplementation of soybean co-products does promote conflicting results. VIOLA and ARIELI (1983) and EL-SAYED (1999) reported that amino acids supplementation in diets containing soybean meal did not improve tilapia performance whereas SHIAU *et al*. (1989) reported that methionine supplementation significantly improved hybrid tilapia growth.

Nile tilapia is a typical omnivore, with long intestines and physiological adaptations to tolerate higher inclusion of dietary plant sources (RODRIGUES *et al*., 2012; GOMINHO-ROSA *et al*., 2014). Soy protein concentrate has higher protein and lower carbohydrate contents than ordinary soybean meal but still showed adequate ADC values for Nile tilapia. However, despite being an omnivore, Nile tilapia showed a decrease in dry matter digestibility when animal protein sources were replaced by SPC. Carnivorous species, which require higher dietary protein contents, such as rainbow trout (MAMBRINI *et al*., 1999) and Atlantic salmon (STOREBAKKEN *et al*., 1998), also showed adequate protein ADC values, indicating the good potential of SPC as a replacement for animal protein sources in fish feeds. Indeed, the composition of alternative animal protein sources can be highly variable (ASKNES and MUNDHEIN, 1997) and usually their ash and phosphorous contents are high, which can hinder the formulation and deteriorate water quality. On the other hand, the process to manufacture SPC removes selectively soluble carbohydrates, some anti-nutritional factors and oligosaccharides (PEISKER, 2001). Therefore, including SPC in fish diets will add other benefits besides serving as an amino acid source.

Liver is considered one of the most important organs for protein metabolism (COWEY, 1994). Glucose can be derived from fat and amino acids in fish metabolism, particularly when there is limited availability of dietary carbohydrates. Excess glucose can turn into fat and accumulate in the liver. Our study demonstrated that the complete replacement of animal protein by SPC protein did not cause deleterious effects on fish liver's histology under light microscopy, such as to promote lipid accumulation, confirming the potential of this ingredient for Nile tilapia.

CONCLUSION

Soy protein concentrate (SPC) can replace poultry by-product meal and fish meal in diets for Nile tilapia without compromising growth performance, protein retention, body composition, liver histology, and protein digestibility. However, SPC supplementation with limiting amino acids, such as methionine and threonine, is advisable since it further increases weight gain and protein digestibility.

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REFERENCES

AOAC - ASSOCIATION OF OFFICIAL ANALITICAL CHEMISTS. 1999 *Official methods of analysis*. 16th ed. 5th Revision. Washington, D.C., 1141p.

ASKNES, A. and MUNDHEIM, H. 1997 The impact of raw material freshness and processing temperature for fishmeal on growth, feed efficiency and chemical composition of Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture*, *149*(1-2): 87-106.

BRASIL 2012 Empresa Brasileira de Pesquisa Agropecuária [*online*]. Seção Soja. Rio de Janeiro. Available at: <https://www.embrapa.br/soja/cultivos/soja1/dados-economicos> Access on: 09 Nov. 2014.

BREMER NETO, H.; GRANER, C.A.F.; PEZZATO, L.E.; PADOVANI, C.R. 2005 Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado da 1,5-difenilcarbazida. *Ciência Rural*, *25*(3): 691-697.

CARGNIN-FERREIRA, E. and SARASQUETE REIRIZ, M. del C. 2008 *Histofisiología de moluscos bivalvos marinos*. Madrid: Consejo Superior de Investigaciones Cientificas. 94p.

CARVALHO, R.A.P.L.F. 2011 *Avaliação da composição de ingredientes selecionados para a substituição da farinha de peixe em dietas para juvenis de* ***Litopenaeus vannamei***. São Paulo. 258f. (Doctoral Thesis. Instituto Oceanográfico da Universidade de São Paulo). Available at: <http://www.teses.usp.br/teses/disponiveis/21/21131/tde-12082011-153008/es.php> Access on: 10 Nov. 2014.

CHO, C.Y. and SLINGER, S.J. 1979 Apparent digestibility measurement in feedstuffs for rainbow trout. In: HALVER, J.E. and TIEWS, K. *Proceedings of the World Symposium of Finfish Nutrition and Fishfeed Technology*. Berlin: Heeneman, p.239-247.

COWEY, C.B. 1994 Amino acid requirements of fish: a critical appraisal of present values. *Aquaculture*, *124*(1-4): 1–11.

DONG, X.H.; GUO, Y.X.; YE, J.D.; SONG, W.D.; HUANG, X.H.; WANG, H. 2010 Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. *Aquaculture Research*, *41*(9): 1356-1364.

EL-SAYED, A.F.M. 1999 Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture*, *179*(1-4): 149-168.

ESPE, M.; HEVRØY, E.M.; LIASET, B.; LEMME, A.; EL-MOWAFI, A. 2008 Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, *Salmo salar*. *Aquaculture*, *274*(1): 132–141.

FERNANDES, V.A.G. 2011 *Perfil nutricional e qualitativo da farinha de vísceras de aves com foco em sua utilização como ingrediente proteico em rações para organismos aquáticos*. Florianópolis. 71f. (Masters Dissertation. Universidade Federal de Santa Catarina - UFSC). Available at: <https://repositorio.ufsc.br/bitstream/handle/123456789/95910/289747.pdf?sequence=1> Access on: 10 Nov. 2014

GOMINHO-ROSA, M.C.; RODRIGUES, A.P.O.; MATTIONI, B.; DE FRANCISCO, A.; MORAES, G.; FRACALOSSI, D.M. 2014 Comparison between the omnivorous jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) on the utilization of dietary starch sources: Digestibility, enzyme activity and starch microstructure. *Aquaculture*, *435*: 92-99.

KAUSHIK, S.J.; CRAVÈDI, J.P.; LALLÈS, J.P.; SUMPTER, J.; FAUCONNEAU, B.; LAROCHE, M. 1995 Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *133*(3-4): 257-274.

KITAGIMA, R.E. 2009 *Digestibilidade da matéria seca, energia, proteína e aminoácidos pelo catfish americano* ***Ictalurus punctatus***. Florianópolis. 53f. (Masters Dissertation. Universidade Federal de Santa Catarina - UFSC). Available at: <https://repositorio.ufsc.br/handle/123456789/93219> Access on: 10 Nov. 2014.

MAMBRINI, M.; ROEM, A.J.; CRAVÈDI, J.P.; LALLÈS, J.P.; KAUSHIK, S.J. 1999 Effects of replacing fish meal with soy protein concentrate

and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, *Oncorhynchus mykiss*. *Journal of Animal Science*, *77*(11): 2990-2999.

NRC - NATIONAL RESEARCH COUNCIL. 2011 *Nutrient Requirements of Fish and Shrimp*. Committee on Nutrient Requirements of Fish and Shrimp. Washington: National Academic Press. 376p.

PARIPATANANONT, T.; BOONYARATPALIN, M.; PENGSENG P.; CHOTIPUNTU, P. 2001 Substitution of soy protein concentrate for fishmeal in diets of tiger shrimp *Penaeus monodon*. *Aquaculture Research*, *32*(s1): 369–374

PEISKER, M. 2001 Manufacturing of soy protein concentrate for animal nutrition. *Cahiers Options Méditerranéennes*, *54*: 103-107.

POPMA, T.J. and LOVSHIN, L.L. 1994 *Worldwide prospects for commercial production of tilapia*. Auburn: Auburn University. 40p.

RIBEIRO, M.J.P. 2012 *Concentrado proteico de soja em rações para tilápia-do-Nilo*. São Paulo. 57f. (Masters Dissertation. Instituto de Pesca , APTA). Available at: <ftp://ftp.sp.gov.br/ftppesca/DissertaManoelJoaquimPerezRibeiro2012.pdf> Access on: 04 Feb. 2014.

RODRIGUES, A.P.O.; GOMINHO-ROSA, M.D.C.; CARGNIN-FERREIRA, E.; DE FRANCISCO, A.; FRACALOSSI, D.M. 2012 Different utilization of plant sources by the omnivores jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition*, *18*(1): 65-72.

SÁ, M.V.C.; SABRY-NETO, H.; NUNES, A.J.P. 2013 Dietary concentration of marine oil affects replacement of fish meal by soy protein concentrate in practical diets for the white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*, *19*(2): 199-210

SHIAU, S.Y.; KWOK, C.C.; HUANG, J.Y.; CHEN, C.M.; LEE, S.L. 1989 Replacement of fishmeal with soybean meal in male tilapia (*Oreochromis niloticus* x *O. aureus*) fingerling diets at a suboptimal protein level. *Journal of the World Aquaculture Society*, *20*(4): 230-235.

STATSOFT, Inc. 2004 STATISTICA (data analysis software system), version 7. [on line] URL: <www.statsoft.com>

STOREBAKKEN, T.; SHEARER, K.D.; ROEM, A.J. 1998 Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. *Aquaculture*, *161*(4): 365-379.

TSUKAMOTO, R.Y. and TAKAHASHI, N.S. 1992 Falta de proteína para ração: estrangulamento da aquicultura no Brasil. *Panorama da Aquicultura*, *14*: 8-9.

VIOLA S. and ARIELI Y. 1983 Nutrition studies with tilapia *Sarotherodon*.: 1. Replacement of fishmeal by soybean meal in feeds for intensive tilapia culture. *Bamidgeh*, *35:* 9–17.

ZAR, J.H. 2009 *Biostatistical Analysis*. 5ª ed. Prentice Hall. 929p.

ZHAO, H.; JIANG, R.; XUE, M.; XIE, S.; WU. X.; GUO, L. 2010 Fish meal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) less than 2 g. *Aquaculture Nutrition*, *16*(6): 648–653.