Processed soybean in diets for pacu (*Piaractus mesopotamicus*)

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ABSTRACT. Changes in development and in physiological parameters of fingerlings of the pacu (*Piaractus mesopotamicus*) fed on diets with high levels of integral crude, extruded, toasted soybean and soybean meal were assessed. The products were evaluated in practical diets for pacu, initially weighing 70 ± 2.19 g, during 82 days. Nine treatments were studied in a 2 × 4 + 1 factorial scheme which corresponded to two fish meal protein replacement levels (50 and 100%) and four soybean products, plus control (100% of fish meal - FM). Development, organosomatic indexes and physiological parameters were evaluated. No differences were reported with regard to weight gain, food intake, food conversion, protein efficiency ratio and specific growth rate. A significant interaction was registered for plasma protein between the evaluated ingredients and replacement percentage of fish meal protein. Results showed that the ingredients assessed did not affect the development of the pacu when compared to fish fed on control diet. Protein retention was favored when toasted soybean and when 50% of the diet's protein fraction from fish meal were employed.

Keywords: antinutritional factors, body composition, physiology.

Soja processada em dietas para pacu (*Piaractus mesopotamicus*)

RESUMO. O objetivo deste estudo foi avaliar alterações no desenvolvimento e em alguns parâmetros fisiológicos de juvenis de pacu (*Piaractus mesopotamicus*), alimentados com dietas contendo altas quantidades de soja integral crua, extrusada e tostada e de farelo de soja. Os ingredientes foram avaliados por 82 dias em dietas práticas para pacus com peso inicial de 70 ± 2,19 g. Foram utilizados nove tratamentos em esquema fatorial 2 × 4 + 1, correspondendo a dois níveis de substituição: 50 e 100% da proteína da farinha de peixe e os quatro tipos de soja mais um controle (100% de farinha de peixe). Foram avaliados o desenvolvimento, índices somáticos e os parâmetros fisiológicos. Não foram observadas diferenças significativas para os parâmetros: ganho em peso, consumo de ração, conversão alimentar, retenção de proteína e crescimento específico. Para proteína plasmática foi observada interação significativa entre os ingredientes avaliados e a porcentagem de substituição da proteína da farinha de peixe. Concluiu-se que os diferentes ingredientes avaliados não tiveram efeito sobre o desenvolvimento do pacu, quando comparado com os que consumiram dieta controle, mas os usos de soja tostada e de 50% de proteína proveniente da farinha de peixe favorecem a retenção de proteína nos peixes.

Palavras-chave: fatores antinutricionais, composição corporal, fisiologia.

Introduction

Fish meal (FM), generally a high-cost product, may be considered of questionable quality when processed from filleting residue, due to the great variation in nutrient contents (Olajuyigbe et al., 2011). In fact, great efforts are being made to explore the use of plant proteins to substitute FM (Gatlin et al., 2007; Tacon & Metian, 2008). Among the various vegetable-based products as potential substitutes for the animal-based protein fraction for fish, soybean meal has received much attention from nutritionists (Lim et al., 2011). Response to replacement of fish meal by soybean meal varies according the species of fish (Zhou, Mai, Tan & Liu,

2005) and the same ingredient may benefit one fish species and adversely affect others (Ostaszewska, Dabrowski, Palacios, Olejniczak & Wieczorek, 2005).

Several researchers have used different thermal treatments to improve the nutritional quality of crude soybean by making milder the antinutritional factors (ANF), which include inhibitors of trypsin, hemagglutinins, goitrogenic substances, antivitamins, isoflavone and phytates (Pedrosa et al., 2012; Torrezan, Frazier & Cristianini, 2010). Stech, Carneiro & Carvalho (2010) evaluated the effect of ANF on soybean meal and on crude and processed soybean in apparent protein digestibility and recommended the use of soybean meal, extruded

soybean and toasted soybean for feed to the pacu (*Piaractus mesopotamicus*). Nevertheless, deleterious effects appear only after a long period and depend on the amount consumed (Viegas, Carneiro, Urbinati & Malheiros, 2008). Thus, the products studied for digestibility should be evaluated in practical diets for a better assessment of their nutritional effects. Since the physiological parameters of *P. mesopotamicus* have been described (Abimorad, Carneiro & Urbinati, 2007; Abreu, Takahashi, Hoshiba & Urbinati, 2009; Takahashi, Abreu, Biller & Urbinati, 2006), the evaluation of these parameters may help understand the effect of fish meal replacement and the consumption of soybean products in different practical diets for the species. Carvalho, Stech & Carneiro (1997) evaluated different soybean cultivars to replace soybean meal in practical diets for the pacu and they concluded that the antinutritional factors evaluated in soybean grains were not enough to compromise the fish's development and metabolic parameters.

P. mesopotamicus of the subfamily Serrasalmidae is mainly reared in the mid-western and southeastern Brazil (Ostrensky, Borghetti & Soto, 2008) but also in China (Lin, Gao & Zhan, 2013) and in the USA (Witmer & Fuller, 2011). The pacu is omnivorous, with specific frugivorous and herbivore feeding habits of an opportunistic pruning type (Dias-Koberstein, Carneiro & Urbinati, 2005). Their earliness, ruggedness, tasty meat, high commercial value, excellent growth and adaptation to artificial feeding provide rearing success in intensive cultivation systems (Abimorad & Carneiro, 2004).

Current study elucidates the effect of high levels of crude and processed soy and soybean meal on performance and physiological characteristics of fingerlings of the pacu (*Piaractus mesopotamicus*).

Material and methods

Crude soybean (CS) under analysis in current assay was produced at the Teaching and Research Farm of the Agricultural and Veterinary Sciences College (UNESP) in Jaboticabal, São Paulo State, Brazil. According to the manufacturer, the toasted soybean (TS) was processed for 40 minutes in an industrial toaster at 120°C. Extruded soybean (ES) was processed without steam injection. The soybean meal (SBM) and fish meal (FM) were purchased on the Brazilian market. The chemical composition of soy products are described in Stech et al. (2010) and the dry matter of FM in current assay comprised 56.73% crude protein, 19.08% lipid, 16.21% mineral matter and 4,456 kcal kg^{-1}, following AOAC (1998).

These products were used in nine diets (Table 1) to replace fish meal (FM) protein at the proportions of 0% (control), 50, and 100% of use. FM supplied 40% of the crude protein in the diet, at the most. The diets were analyzed for their chemical composition, according to AOAC (1998).

The treatments in this study were:

FM100: FM supplied 40% of the crude protein in the diet (control);

ES100: 100% of FM protein was replaced by ES;

TS100: 100% of FM protein was replaced by TS;

CS100: 100% of FM protein was replaced by CS;

SBM100: 100% of FM protein was replaced by SBM;

ES50: 50% of FM protein was replaced by ES;

TS50: 50% of FM protein was replaced by TS;

CS50: 50% of FM protein was replaced by CS;

SBM50: 50% of FM protein was replaced by SBM.

Table 1. Experimental diet ingredients and analyzed composition.

Ingredient (g)	Diets (g kg^{-1})								
	FM100	ES100	TS100	CS100	SBM100	ES50	TS50	CS50	SBM50
Extruded Soybean (ES)	0.0	269.0	0.0	0.0	0.0	134.5	0.0	0.0	0.0
Toasted Soybean (TS)	0.0	0.0	278.0	0.0	0.0	0.0	135.4	0.0	0.0
Crude Soybean (CS)	0.0	0.0	0.0	270.8	0.0	0.0	0.0	135.4	0.0
Soybean Meal (SBM)	0.0	0.0	0.0	0.0	262.0	0.0	0.0	0.0	131.0
Fish Meal (FM)	197.8	0.0	0.0	0.0	0.0	99.0	99.0	99.0	99.0
Wheat Bran	99.0	25.0	23.6	236.5	20.0	22.1	15.85	16.08	11.52
Corn Flour	321.4	146.0	135.0	136.7	120.0	180.0	241.0	230.2	253.1
Rice Bran	50.0	50.0	56.0	40.0	40.0	76.4	46.0	50.0	32.5
Soybean Oil	20.4	0.0	0.0	16.0	55.0	9.2	09.5	17.8	38.6
Yeast	306.4	280.0	290.0	295.0	318.0	274.9	306.0	301.8	325.6
Mineral and vitamin mix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Analyzed composition (g kg^{-1}, dry basis)[2]									
Dry Matter	889.50	885.94	895.57	888.91	886.93	887.95	892.09	889.22	888.16
Crude Protein	260.03	259.74	260.00	260.05	259.66	260.00	259.95	260.02	260.00
Lipid	78.85	81.29	82.92	79.97	76.46	81.77	78.71	79.44	77.18
Mineral	61.03	45.63	42.69	41.50	45.40	55.53	51.31	51.69	52.20
Crude Fiber	22.07	56.61	40.02	40.24	27.95	41.94	30.27	31.44	23.61
NFE	467.51	442.66	469.94	467.15	477.46	448.71	471.85	466.63	475.17
Gross Energy[3]	3.549	3.542	3.733	3.654	3.666	3.544	3.633	3.610	3.630

[1]Composition per kg: (vit. A: 176,000 IU; vit. D3: 40,000 IU; vit E: 500 mg; vit. K3: 100 mg; vit. B1: 36 mg; vit. B2: 200 mg; vit. B6: 50 mg; vit. B12: 560 mcg; niacin: 700 mg; biotin: 3 mg; pantothenic acid: 500 mg; folic acid: 30 mg; choline: 20 mg; Fe: 1,100 mg; Cu: 300 mg; Mn: 1,800 mg; Zn: 1,200 mg; I: 24 mg; methionine: 20 g; Se: 3 mg; Ca: 176 g; P: 68 g; Na: 23 g; Cl: 36 g; growth promoter: 2 g; antifungal: 200 mg; BHT: 1 g; energetic, protein vehicle (qs): 1 kg. [2]Proximate compositions were determined according to AOAC (2012). [3]kcal g^{-1}

A performance trial and analyses were conducted at the Aquaculture Center of the Universidade Estadual Paulista (CAUNESP; Jaboticabal, São Paulo State, Brazil) and procedures were approved by the local Animal Ethics Committee. Pacu juveniles were acclimated to laboratory conditions for 4 weeks and the experiment was carried out during 82 days. One hundred and eighty juvenile pacus, with a mean initial weight of 70 ± 2.19 g, were randomly distributed in thirty-six 150-L cement tanks. The tanks were supplied with water from an artesian well at a renewal rate of approximately 20 x per day. The water's physicochemical analyses in the experimental units showed little variation during the period under analysis. The pH values were maintained at approximately 7.5 ± 0.13, and temperature varied between 28.0 ± 0.9°C in the morning and 28.5 ± 0.8 in the afternoon. Total alkalinity and dissolved oxygen were 2.04 ± 0.05 mg L^{-1} and 5.97 ± 0.43 mg O$_2$ L^{-1}, respectively. Feed was provided twice a day to apparent satiation, at approximately 8h00 am and 6h00 pm.

At the beginning of the experiment, 20 juveniles from the starting population and four from each tank at the end of the experimental period were sampled to analyze their body composition. The fish fasted for 24h to empty the digestive tract and, after the last biometric determination, were euthanized by an overdose of benzocaine (100 mg L^{-1}), frozen and ground. The ground material was dried in a forced-air circulation oven at 55°C until constant weight for crude protein analyses (AOAC, 1998).

Assessment of the diets in 82 days of the experimentation period was done by calculating:

$$\text{Absolute weight gain}\,(WG) = \frac{\text{final body weight}\,(g) - \text{initial body weight}\,(g)}{\text{number of fish}}$$

$$\text{Feed conversion}\,(FC) = \frac{\text{feed intake}\,(g)}{\text{weight gain}\,(g)}$$

$$\text{Protein efficiency ratio}\,(PER) = 100\,x\,\frac{\text{weight gain}\,(g)}{\text{protein intake}\,(g)}$$

$$\text{Specific growth rate}\,(SGR) = 100\,x\,\frac{\ln \text{final body weight} - \ln \text{initial body weight}}{\text{days}}$$

$$\text{Protein retention}\,(PR) = 100\,x\,\frac{\text{final fish body protein}\,(g) - \text{initial fish body protein}\,(g)}{\text{protein intake}\,(g)}$$

where:

$$\text{Protein intake} = \text{feed intake}\,x\,\%\,\text{Crude Protein in diet}$$

On the sampling day and after a 24h-fast, four fish from each tank were harvested and immediately anaesthetized with benzocaine (100 mg L^{-1}). The blood was sampled by puncturing the caudal vein

and transferred to 1.5 mL centrifuge microtubes divided into two groups (with and without fluoride anticoagulant). Next, the abdominal cavity was opened to remove the liver, which was weighed, stored in cell cultivation plates, frozen in dry ice and maintained at -20°C to determine hepatic glycogen dosage (Sigma Trinder Kit) and total liver lipids (Bligh & Dyer, 1959). Abdominal fat and the digestive tract were weighed separately. The hepato-somatic index (HIS), visceral fat somatic index (VFSI), and digestive somatic (DSI) indexes were obtained based on the relationships between their weights (liver weight, visceral fat weight, or digestive tract weight from the esophagus to the anus, respectively) and the animal's total body weight. The blood containing fluoride was used to determine hematocrit (micro-hematocrit method) and glycaemia (King & Garner, 1947). Another fraction of the blood was removed for plasma separation to determine total plasma protein (Gornall, Bardawill & David, 1949) and plasma triglycerol (Labtest Diagnostic Kit, Lagoa Santa, Minas Gerais State, Brazil).

Performance, body composition and physiological results were analyzed according to a completely randomized design with nine treatments, in a 4 × 2 + 1 factorial scheme, consisting of four products, two protein and fish meal replacement levels, and control, with four replicates. After confirming normality by the Cramer-von Mises method and homoscedasticity by the Levene test, data were analyzed by two-way ANOVA and means were assessed by Tukey's test ($p < 0.05$).

Results and discussion

Studies on Brazilian products developed by Fernandes, Carneiro & Sakomura (2001) showed that the addition of different whole soybean levels in feeds for pacu juveniles did not induce alterations on production performance and fish survival parameters. The above demonstrated the potential of soybean as a main protein concentrate in diets for these species.

No significant effects ($p > 0.05$) were reported on the soybeans used or even on FM replacement levels with regard to weight gain, food intake, food conversion, food efficiency and food efficiency rates (Table 2). Different authors have shown negative effects on weight gain and on the use of nutrients when crude soybean and soybean meal were added in diets for many fish species, generally from temperate climate (Barrows, Stone & Hardy, 2007; Collins et al., 2012; Lim et al., 2011; S. Lin & Luo, 2011). The tropical omnivorous pacu, featuring specific frugivorous-herbivorous feeding habits, is probably less

demanding in terms of animal protein (Fernandes et al., 2001) and less sensitive to ANF normally present in legumes (Ostaszewska et al., 2005; Stech et al., 2010). In current study, the inclusion of soybean in the diets did not impair feed intake when compared with the treatment containing FM alone. The above discards the possibility that pacu is sensitive to the characteristic flavor in soybean. The same observation was made by Venou, Alexis, Fountoulaki & Haralabous (2006) who evaluated SBM and SBM extruded in diets for *Sparus aurata*, and by S. Lin & Luo (2011) who reported on the tilapia an increase in diet ingestion when there was an increased replacement of fish meal by soybean meal.

All diets studied showed high energy rates due to the lipid contents in ES and ST (Stech et al., 2010) and the consequent need for adding soybean oil in some treatments to reduce the differences in diet composition, perhaps with limited intake and better fish growth. However, Signor et al. (2010) did not report performance improvements when there was a decrease in energy protein ratio in diets for the pacu. Fernandes et al. (2001) reported similar performance results under the same experimental conditions.

Diets containing TS or feeds containing 50% of their protein from FM showed better means than protein retention results ($p < 0.01$) (Table 2). Hansen, Rosenlund, Karlsen, Koppe & Hemre (2007) verified that the replacement of fish meal with plant proteins in diets for the Atlantic cod (*Gadus morhua*) reduced protein retention, as registered in current study. These authors explained that reduced retention rates may be partly explained by unbalanced amino acid concentrations in diet, resulting in increased protein turnover. The fact that TS showed higher mean protein retention rates must be associated with greater amino acid availability. Thermal treatment seems to be the process that most affects the nutritional quality of soybean protein. Heating makes the proteins become more susceptible to the

hydrolysis of enzymes in the product, with activity on lipids and carbohydrates bound to the protein (Lentle & Janssen, 2011). Stech et al. (2010) reported that although there was no difference in the digestibility of the protein fraction in TS, ES, SBM, the employment of these ingredients for an 82-day period in current study showed differences in protein retention between TS and ES and FM. This is due to the effect of intake of antinutritional factors for a long time, especially tannin that forms complexes with proteins and digestive enzymes and, according Stech et al. (2010), is lower in TS than in ES, CS and SBM.

Table 2 does not show any difference ($p > 0.05$) in HSI and VFSI to soybeans used and to FM, or even on FM replacement levels. Ye, Liu, Wang & Wang (2011) reported a significant increase in HSI with increased dietary SBM levels in FM replacement in diets for the juvenile Japanese flounder. The authors correlated this fact with relatively high carbohydrates from SBM. In the case of the pacu, HIS increases with increasing energy content of the diet. Increased HIV is related to increase in protein and carbohydrate contents in the diet (Abimorad et al., 2007). The experimental diets were formulated to contain the same amounts of nutrients and this reflected in non-significant differences in HIS and VFSI. Higher hemagglutinatin activity present in the crude soy (Stech et al., 2010) is not sufficient to decrease the concentration of insulin in the blood and the consequent change of HIS and VFSI rates. Proteinase inhibitors in some animals cause pancreatic hypertrophy but it is not clear whether this also takes place in fish (Krogdahl, Penn, Thorsen, Refstie & Bakke, 2010). In current study, the ingested trypsin inhibitors were not apparently sufficient to produce hepatocyte hyperplasia since no HIS alterations were observed among the treatments.

Table 2. F values and average of the weight gain (WG, g day^{-1}), food intake (FI, g day^{-1}), food conversion (FC, g g^{-1}), protein efficiency ratio (PER, g g^{-1}), specific growth rate (SGR, % day^{-1}), protein retention (PR, g g^{-1}), hepato-somatic index (HIS, %), visceral fat somatic index (VFSI, %) and digestive somatic index (DSI, %) of pacu juveniles fed on different sources of soybean and proportional replacement of fish meal.

F value	WG	FI	FC	PER	SGR	PR	HSI	VFSI	DSI
Soybean products (S)	0.99[ns]	0.71[ns]	0.82[ns]	0.93[ns]	0.94[ns]	16.21**	0.87[ns]	0.81[ns]	3.73**
FM × S	0.00[ns]	0.00[ns]	0.06[ns]	0.18[ns]	0.00[ns]	2.81[ns]	2.38[ns]	0.05[ns]	0.01[ns]
Replacement level (L)	0.07[ns]	0.08[ns]	0.01[ns]	0.15[ns]	0.11[ns]	11.30**	0.02[ns]	0.10[ns]	0.65[ns]
S×L interaction	0.87[ns]	0.90[ns]	0.36[ns]	0.86[ns]	0.98[ns]	0.94[ns]	2.01[ns]	0.59[ns]	1.92[ns]
Treatments	0.70[ns]	0.61	0.45[ns]	0.71[ns]	0.73[ns]	8.20**	1.38[ns]	0.54[ns]	2.21[ns]
Effect of Feedstuff									
Extruded soybean (ES)	0.61	1.57	2.70	1.53	0.64	29.27[b]	1.12	2.54	3.37[b]
Toasted soybean (TS)	0.55	1.39	2.79	1.40	0.59	42.15[a]	1.03	2.74	3.64[ab]
Crude soybean (CS)	0.53	1.54	3.23	1.32	0.57	24.06[b]	1.17	2.24	3.78[ab]
Soybean meal (SBM)	0.43	1.34	3.11	1.26	0.37	26.59[b]	1.15	2.47	4.08[a]
Fish meal (FM)	0.54	1.46	2.89	1.46	0.54	25.50[ab]	1.27	2.58	3.69[ab]
Protein fish meal replacement									
100%	0.54	1.48	2.94	1.41	0.58	27.20[b]	1.12	2.53	3.73
50%	0.52	1.44	2.94	1.36	0.57	33.87[a]	1.11	2.46	3.71

Means designated by the same letter in the column do not differ ($p > 0.05$) by Tukey's test. [ns] not significant by Tukey's test ($p > 0.05$). ** Significant at 1% probability by Tukey's test ($p < 0.01$).

Since DSI results showed significant differences (p < 0.01) for the soybeans studied, the fish fed on diets with SBM showed heavier digestive tracts than fish fed on ES. Bakke-McKellep et al. (2007) observed that *Salmo salar* fed on SBM had a lower DSI when compared to fish fed on FM and correlated increased DSI with peristaltic activity. The same correlation may be registered in current study because the fiber contents in the diets ES100 and ES50 were higher than SBM100 and SBM50. Olsen et al. (2007); (Zhou et al., 2005) failed to register any effect of total FM replacement by plants in the diet on the relative gut weight of the Atlantic cod and cobia juveniles. The same occurred in the pacu.

According to Wang, Hung & Randall (2006), alterations in the digestive tract and on the amount of visceral fat occur when the animals undergo some type of food restriction. Observing the results of HIS and VFSI, DHI and WG, the diets containing SBM produced lower muscle tissue synthesis. The above shows that the antinutritional factors present in SBM (Stech et al., 2010) may affect carcass yield of pacu for a long time, even with no statistical difference (p > 0.05) between diets with soybean (ES, TS, CS, SBM) and control (FM). The FM produced in Brazil is not generally considered of high quality because it is drawn up on fish waste (Abimorad & Carneiro, 2004), and thus cannot be compared with that used in studies developed in other parts of the world, or rather, with higher protein and amino acid percentages. In fact, the analysis of variance showed no statistical difference (p > 0.05) between control diet and other diets for all analyzed parameters.

Blood parameters show animals' health and nutritional status (Tavares-Dias, Oliveira-Junior, Silva, Marcon & Barcellos, 2009). Zhou et al. (2005) reported differences in plasma, glucose and triglyceride concentration in juvenile cobia fed on diets with different soybean meal replacement levels. According to these authors, fishes fed on diets containing soybean protein replacement level exceeding 400 g kg^{-1}, had a significant decrease in hemoglobin, hematocrit and red blood cell. Lim et al. (2011) found that hematocrit and hemoglobin significantly decreased when the fish meal was replaced by 45 or 60% soybean meal. However, Silva-Carrillo, Hernández, Hardy, González-Rodríguez & Castillo-Vargasmachuca (2012) suggested that hematological rates were not useful indicators of diet quality as they were related to SBM level. Lochmann et al. (2009) concluded that there were no obvious antinutritional effects of alternative feeds on body composition, hematology and nonspecific immune response of *Colossoma macropomum* and *Piaractus brachypomus*. Results of analyses of variance for the physiological analyses of current study did not show any difference (p > 0.05) on hematocrit, plasma triglycerides, total liver lipid, glycogen and glycaemia in relation to the factors analyzed (Table 3). Average hematocrit rates of pacu indicated that the fish were under similar environmental conditions and were not in a state of malnutrition or supposedly diseased or that the ANF in the ingredients evaluated were able to change this characteristic.

High total lipids, triglycerides, and glycogen values in the liver and low plasma protein levels (Table 4) were observed. The above suggested an imbalance of the protein-energy relationship in all treatments, which caused an energy storage accumulation in fat and carbohydrates.

Table 3. Average and F values to hematocrit (Hm, %), plasma triglycerides (PT, mg dl^{-1}), glycaemia (Gl, mg dl^{-1}), hepatic glycogen (HG, mg g^{-1}) and total liver lipids (TLL, mg g^{-1}) for juveniles of pacu fed on different sources of soybean and replacement proportion of fish meal.

F value	Hm	PT	Gl	HG	TLL
Soybean products (S)	0.93[ns]	1.43[ns]	0.50[ns]	0.88[ns]	0.26[ns]
FM × S	0.01[ns]	0.79[ns]	2.63[ns]	0.07[ns]	0.10[ns]
Replacement level (L)	0.13[ns]	0.33[ns]	0.02[ns]	0.35[ns]	0.17[ns]
S×L interaction	0.25[ns]	0.95[ns]	0.33[ns]	0.04[ns]	0.26[ns]
Treatments	0.46[ns]	1.03[ns]	0.65[ns]	0.40[ns]	0.23[ns]
Effect of feedstuff					
Extruded soybean (ES)	38.00	184.47	78.78	156.3	133.1
Toasted soybean (TS)	39.13	186.13	75.77	158.3	128.2
Crude soybean (CS)	37.88	194.92	83.79	190.3	128.4
Soybean meal (SBM)	40.75	220.47	78.74	166.4	146.4
Fish meal (FM)	38.80	215.10	90.70	174.0	142.0
Protein fish meal replacement					
100%	39.19	200.48	79.63	172.7	137.5
50%	38.69	192.51	78.90	162.9	130.5

[ns] Not significant by Tukey's test (p > 0.05).

Table 4. Partitioning of significant interaction (p < 0.05) for plasma protein (mg dl⁻¹) of pacu juveniles fed on different sources of soybean and replacement proportion of fish meal.

Soybean products	Fish meal protein replacement	
	50%	100%
Extruded soybean (ES)	0.18 [a]	0.07 [b]
Toasted soybean (TS)	0.12 [a]	0.11 [a]
Crude soybean (CS)	0.09 [a]	0.16 [a]
Soybean meal (SBM)	0.17 [a]	0.13 [a]

Means given by the same letter in the line do not differ (p > 0.05) by Tukey's test.

According to Bicudo, Sado & Cyrino (2009), plasma triglyceride concentrations were reported to increase as dietary lipid levels increased, but no significant effect of protein and energy levels, or their interaction, was observed in their analysis. The same seemed to have happened in current assessment. Circulating glucose rates were within the limits observed by Bicudo et al. (2009) for the fish species and none of the treatments changed this variable. Since the decrease in circulating glucose levels expected for fish that consumed diets containing hemagglutinins (CS) was not observed, the pacu's ability in overcoming the insulin-mimetic action of this antinutritional factor was demonstrated.

In the case of plasma protein, a significant interaction (p < 0.05) was observed between soybean types and replacement levels (Table 4). The partitioning analysis for this interaction showed that averages FM 50% replacement level were higher than those for the 100% level only when extruded soybean diet was used (p < 0.05). Plasma protein showed very low mean rates when compared to those described for pacu (Bicudo et al., 2009; Takahashi, Biller & Urbinati, 2011). The low level of plasma protein in current study could be an indication of low tissue protein synthesis and, therefore, of low availability of enzymes involved in the metabolic pathways of utilization of hepatic storage (fat and carbohydrates) and transportation of liver lipids, causing accumulation.

Conclusion

Under current study's experimental conditions, it may be concluded that the use of high amounts of *in natura* or processed soybean, when compared to diets with fish meal, did not affect the growth performance of pacu. However, protein retention was favored when toasted soybean and when 50% of the diet's protein fraction from fish meal were employed.

Acknowledgements

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