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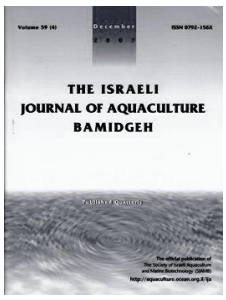

Effects of Dietary Soy Protein Concentrate on Growth Performance, Digestion, and Protein Metabolism of Juvenile Darkbarbel Catfish *Pelteobagrus vachelli*

Yinghao Yang, Qinghui Ai*, Kangsen Mai, Wei Xu, Wenbing Zhang, Xiaojie Wang and Jipeng Xue

The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao, China

Key words: *Pelteobagrus vachelli,* soy protein concentrate, growth, digestion, protein metabolism

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Abstract

A 10-week feeding trial in a recirculation rearing system was conducted to investigate the effects of dietary soy protein concentrate (SPC) levels on survival, growth, digestion, and protein metabolism of juvenile darkbarbel catfish *Pelteobagrus vachelli*. The results demonstrated that survival and whole-body composition were independent of dietary treatments. Feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), apparent digestibility coefficients, digestive enzymes (pepsin and trypsin) activity and protein metabolism enzymes (alanine aminotransferases) decreased with increasing dietary SPC. The diet with 60% SPC was least cost effective. Results suggest that SPC could replace 60% or less fish meal protein without negatively influencing the growth of juvenile *Pelteobagrus vachelli*.

* Corresponding author. e-mail: qhai@ouc.edu.cn

Introduction

Fish meal (FM) is a major protein source in aquafeeds (Tacon and Metian 2008). However, with the expansion of aquaculture, increasing demand, uncertain availability, and high prices have increased the need to search for low-cost on-farm prepared alternative protein sources (Ai et al. 2006; Salze et al. 2010;). Of all alternative protein sources, soy protein concentrate (SPC) has the greatest potential for higher feed intake, better digestion, and balance of essential amino acids. Compared to FM, SPC produces higher and more stable yields at a lower cost.

SPC is produced through aqueous ethanol or methanol extraction of defatted soy flakes, which typically contain 65-70% crude protein (Hardy 2008). Many studies have shown success in partial (25-75%) or total replacement of FM with SPC in fish diets (Berge et al. 1999; Mambrini et al. 1999; Ustaoglu and Rennert 2002 ; Aragão et al. 2003; Deng et al. 2006; Romarheim et al. 2006),.

Pelteobagrus vachelli (darkbarbel catfish) is an important freshwater aquaculture species in China. Culture of this species has rapidly increased due to its delicious meat and high market value. To our knowledge, there have been no studies on plant protein replacement in the feed for *P. vachelli* juveniles. The main objective of the present experiment was to investigate the effects of dietary SPC on growth performance, digestion, and protein metabolism of juvenile *P. vachelli*.

Materials and methods

Experimental diets. Six isonitrogenous (crude protein 39%) and isoenergetic (19.5 kJ/g) diets replacing 0, 20, 40, 60, 80 and 100% FM protein with SPC were formulated to contain 0%, 9.81%, 19.61%, 29.41%, 39.22% and 49.02% SPC levels, which were abbreviated to SPC 0%, SPC 20%, SPC 40%, SPC 60%, SPC 80% and SPC 100%, respectively (Table 1) (Yang et al. 2011). In addition, 400 mg/kg yttrium oxide (Y2O3, Fluka Chemicals®) was used as an inert tracer in each diet to determine apparent digestibility of nutrients. The essential amino acid compositions for each diet are shown in Table 2.

Table 1. Formulation & chemical composition (% dry matter) of experimental diets (Yang et al. 2011)

Ingredient	*Diet abbreviation*					
	SPC100%	*SPC80%*	*SPC60%*	*SPC40%*	*SPC20%*	*SPC0%*
Fish meal	0.00	9.24	18.47	27.71	36.95	46.18
SPC	49.02	39.22	29.41	19.61	9.81	0.00
Wheat gluten	25.00	25.00	25.00	25.00	25.00	25.00
Wheat bran	13.00	13.00	13.00	13.00	13.00	13.00
Fish oil	4.50	3.88	3.28	2.67	2.05	1.44
Soybean oil	1.10	1.25	1.38	1.52	1.66	1.80
Lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Attractant	0.50	0.50	0.50	0.50	0.50	0.50
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05
Y_2O_3	0.04	0.04	0.04	0.04	0.04	0.04
Microcrystallinecellulose	0.86	1.90	2.90	3.98	5.07	6.06
Proximate composition (% dry matter)						
Crude protein (%)	39.20	39.13	39.21	39.30	39.17	39.40
Crude lipid (%)	8.60	8.97	9.04	9.03	8.79	9.04
Ash (%)	6.35	7.33	8.17	9.11	10.21	11.29
Gross energy (kJ/g)	19.65	19.71	19.54	19.59	19.42	19.46

Fish meal: crude protein 74.3% dry matter, crude lipid 7.5% dry matter; SPC: crude protein 70.1% dry matter, crude lipid 2.4% dry matter; Wheat gluten: crude protein 16.4% dry matter, crude lipid 1.0% dry matter; Wheat bran: crude protein 18.6% dry matter, crude lipid 2.5% dry matter.

Mineral premix (mg or g/kg diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$(1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1,200 mg; Ca $(H_2PO_4)2 \cdot H_2O$, 3,000 mg; NaCl, 100 mg; Zoelite, 15.448 g.

Vitamin premix (mg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine-HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2000 mg, ethoxyquin, 150 mg, microcrystalline cellulose, 14.52 g.

Attractant: glycine and betaine. Data represents mean ± SEM (n = 3).

Table 2 Essential amino acid composition of the experimental diets (% dietary protein)

Essential amino acid	*Diet abbreviation*					
	SPC100%	*SPC80%*	*SPC60%*	*SPC40%*	*SPC20%*	*SPC0%*
Arginine	7.1±0.04	6.6±0.05	6.2±0.04	5.7±0.01	5.2±0.01	4.7±0.10
Histidine	2.7±0.12	2.3±0.03	2.0±0.07	1.6±0.03	1.3±0.02	0.9±0.04
Lysine	5.4±0.03	5.4±0.09	5.3±0.11	5.4±0.10	5.4±0.04	5.3±0.04
Leucine	8.1±0.02	7.7±0.03	7.2±0.03	7.0±0.04	6.4±0.02	5.9±0.01
Isoleucine	4.6±0.05	4.4±0.01	4.1±0.07	4.0±0.08	3.7±0.03	3.4±0.04
Methionine	1.3±0.04	1.5±0.03	1.7±0.06	1.8±0.06	2.0±0.05	2.0±0.13
Phenylalanine	5.3±0.12	4.9±0.11	4.7±0.05	4.1±0.07	3.7±0.04	3.5±0.01
Threonine	3.7±0.01	3.6±0.01	3.6±0.03	3.4±0.09	3.3±0.06	3.3±0.07
Valine	4.8±0.02	4.7±0.04	4.7±0.07	4.4±0.05	4.3±0.11	4.1±0.08

No tryptophan was detected because of acid hydrolysis.
Data represent mean ± SEM (n = 3).

Ingredients were ground into 246-μm fine powder particles. All the ingredients were thoroughly mixed with menhaden fish oil, and water added to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China) and dried for ~ 12h in a ventilated oven at 45°C. After drying, the feed pellets were broken and sieved into proper pellet size (1.5×3.0mm), and stored at -20°C until use (Yang et al. 2011).

Feeding trial procedures. Juvenile *P. vachelli* were obtained from a commercial farm in Meishan, Sichuan, China. Prior to the experiment *P. vachelli* juveniles were reared in a 300 l fiberglass tank with dechlorinated freshwater from a holding tank. The water was circulated through biological filters. The fish were acclimated for two weeks during which they were fed the control diet.

Prior to onset of the experiment, the fish were not fed for 24 h. They were weighed after being anesthetized with tricaine methanesulfonate (1:10,000) (MS-222, Shanghai Reagent Corp., China at 0.1mg/l). Fish of similar sizes (1.54 ± 0.01g) were randomly distributed into 18 tanks each stocked with 40 fish and held under natural photoperiod conditions throughout the trial. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice daily (07:00 and 18:00). The feeding trial lasted for 10 weeks. During the rearing period, water temperature, dissolved oxygen, and pH values were maintained at 26±1°C, 7-9 mg/L and 6.8-7.6, respectively. Total ammonia-nitrogen was less than 0.2mg/l and nitrite level was below 0.09 mg/l.

Sample collection. At termination of the experiment, fish were not fed for 24 h before harvest. Total number and body weight of fish in each tank was recorded and measured. Five fish per tank were collected and stored at -20°C to determine whole-body proximate composition. Another five fish from each tank were anesthetized with MS-222, and blood samples were collected from the caudal vein using a 27 gauge needle, a 1-ml syringe, and allowed to clot at room temperature for 4 h. Following centrifugation (4000×g, 15min, 4°C), the serum was removed and frozen at -80°C until assayed. The liver, stomach, and intestinal tract were dissected from three fish from each tank with a 0.01M phosphate-buffered saline (PBS, pH 7.0) and stored frozen at -20°C.

The distal intestines (DI), from the region of increase in intestinal diameter and presence of visible folds to the anus were removed from another three fish and were fixed by immersion in Serra's solution (ethanol/formol/acetic acid, 6/3/1).

To determine the apparent digestibility coefficients (ADCs) for protein, after the 10-week feeding period, 20 fish from each tank were stripped for feces by gently squeezing the hind gut of randomly selected fish 6 h after feeding. The hind gut content was collected in a plastic bowl on ice, stabilized with ethoxyquin solution (400 mg/l), and immediately frozen at -20°C. Feces were kept frozen and then freeze dried prior to analysis.

Analysis of diets and fish body composition. At the initiation and the end of the experiment a sample of 20 and 5 fish per tank respectively were collected, frozen (-20^0C) and stored for proximate analysis of the carcass composition. Proximate analysis of feed ingredients, experimental diets, and fish body were performed by standard

methods of AOAC (1995). Samples of diets and fish body were dried to a constant weight at 105°C to determine the dry matter content. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method (Kjeltec 2300 Protein Analyzer, Denmark), lipid by ether extraction using Soxhlet (B-801, Switzerland), ash by combustion at 550°C, and energy by an adiabatic bomb calorimeter (PARR1281, USA). The content of essential amino acids (except for methionine) in diets was determined by high performance Biochrom 30 amino acid Auto-analyser (Biochrom Ltd®, England) after being hydrolyzed for 24 h with hydrochloric acid under 110ºC. Methionine was determined according to the method of Mai et al. (2006) using reverse-phase high-performance liquid chromatography (HPLC, HP1100, USA).

Apparent digestibility analysis. Samples were digested in perchloric acid at a ratio of 1:50 (w/v). They were then diluted to 100 ml using deionized water and yttrium and phosphorus determined by inductively coupled plasma-atomic emission spectrophotometer (ICP-OES, VISTA-MPX, VARIAN, USA). Apparent digestibility coefficients (ADC) were estimated according to the formula:

ADC of nutrients (%) = 100−100 × ((Yfeed / Yfaeces) × (Nfaeces / Nfeed))

where Yfeed=Yttrium oxide in feed, Yfaeces =Yttrium in feces, Nfeces =nutrient in feces, Nfeed =nutrient in feed. All data were based on calculated dry weight of the samples.

Protein digestive enzymes assay. The stomach and gut samples were accurately weighed, then homogenized in ice-cold distilled water, 1:5 (w/v). Following centrifugation (1800×g, 30min, 4°C), the supernatants were removed and kept at 4°C for analysis. Pepsin (E.C.3.4.23.1) was analyzed at pH 2.0 using bovine hemoglobin as substrate dissolved in HCl (1M) solution (pH 2.0). Pepsin activity was expressed as specific activity with 1 U representing 1 mM equivalent of tyrosine liberated per minute per mg of protein at 37°C. Trypsin (E.C.3.4.21.4) activity was assayed at 25°C using BAPNA (N-α-benzoyl-dl-arginine-p-nitroanilide) as substrate in 50 mM Tris–HCl, 20 mM CaCl2 buffer, pH 8.2. One unit of trypsin per ml (U) was defined as 1 μmol BAPNA hydrolyzed per min per ml of enzyme extract at 407 nm (Holm et al. 1988).

Protein metabolism enzymes assay. Crude extract of liver for assaying protein metabolism enzyme activity were obtained by homogenization of frozen tissue in ice-cold 0.7% saltwater. Following centrifugation (3200×g, 20 min, 4°C), aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) in liver supernatants and hematoplasma were measured using specific analytical procedures and commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China). All assays were conducted within 24 h of extraction.

Histological analysis. After 36 h, all samples were dehydrated and embedded in paraffin according to standard histological procedures. A series of sagittal and cross sections (5-7μm) were cut from each paraffin block, mounted on glass slides, air dried, and stained with hematoxylin and eosin. The sections were examined and photographed under an Olympus BX 51 microscope (Nikon Corporation, Japan) equipped with an Olympus Digital Sight BP 70 camera (Nikon Corporation, Japan).

Cost analysis. The cost of feed and sale price of fish were the only economic criteria used. They were based on the current market cost of feed and market value per kilogram of fresh fish in China at the time of the experiment. The evaluations were calculated as follows:

Cost of feed (US) = Cost of ingredients + Processing + Labor

Profit index (US) = Value of fish / Cost of feed.

Calcuations & statistical methods. The following variables were calculated:

Survival (%) =100×(final amount of fish) / (initial amount of fish)

Specific growth rate (SGR) = (LnWt−LnW0) × 100/t

Feed intake (FI) = 100 × I × 2 /((Wt+ W0) × t)

Feed Conversion ratio (FCR) = feed offered (g) / biomass gain (g).Where Wt and W0 were final and initial body weight, respectively; t was duration of experimental days; I (g) was feed intake as dry matter.

All data were subjected to analysis of variance using SPSS17.0 for Windows. Differences among the means were tested by Tukey's multiple range tests. The level of significance chosen was $P < 0.05$.

Results

Survival and growth performance. Survival rate ranged from 97.5-100%, and was independent of dietary treatments (Table 3). There were no significant differences in FI (from 1.15 to 1.18 %/100g/day) among fish fed the diets SPC 0, 20, 40 and 60% and fish fed the diets SPC 80 and 100% both of which were significantly lower than the control diet ($P < 0.05$). SGR of fish decreased with increasing dietary SPC, and SPC 20% showed the highest SGR. However, there were no significant differences in SGR among fish fed the diets SPC 0, 20, 40 and 60%. FCR showed a similar trend as FI. FCR was significantly lower compared to the control group when the substitution level was 80% or more ($P < 0.05$) (Table 3).

Table 3 Growth performance and survival of *Pelteobagrus vachelli* (darkbarbel catfish) fed the diets with graded levels of SPC

Diet ab.	*Growth response*					
	IW(g)	*FW(g)*	*SGR(%/d)*	*Survival(%)*	*FCR*	*FI(%100g/d)*
SPC100%	1.53±0.04	10.73±0.56^{a}	2.68±0.08^{a}	99.2±0.00	1.29±0.06^{a}	1.07±0.07^{a}
SPC80%	1.52±0.05	14.76±0.43^{b}	3.25±0.04^{bc}	98.3±0.83	1.26±0.43^{ab}	1.12±0.02^{ab}
SPC60%	1.56±0.02	16.25±0.99^{bcd}	3.43±0.07^{bcd}	100.0±2.50	1.23±0.77^{bc}	1.15±0.06^{bc}
SPC40%	1.56±0.05	17.93±0.31^{cd}	3.49±0.07^{bcd}	97.5±1.67	1.20±0.55^{c}	1.15±0.02^{bc}
SPC20%	1.50±0.03	18.06±0.08^{cd}	3.55±0.04^{d}	100.0±0.83	1.20±1.07^{c}	1.16±0.06^{bc}
SPC0%	1.56±0.01	18.44±0.72^{d}	3.52^{c}±0.05^{d}	98.3±0.00	1.20±0.51^{c}	1.18±0.07^{c}

IW: Initial weight; FW: Final weight; SGR: Special growth rate; FCR: Feed Conversion ratio; FI: Feed intake ratio.
Data represent mean ± SEM (n = 3).
Values in the same column with the same superscript letters are not significantly different ($P > 0.05$).

Whole-body composition. The whole-body protein showed a decreasing trend (from 15.2-14.0%) and lipid exhibited an increasing trend (from 12.2-13.4%) with increasing dietary SPC. However, there were no significant differences between the dietary treatments (Table 4). Also no significant difference was found in moisture content (69.1-71.0%) among dietary treatments (Table 4).

Table 4 Proximate composition (% wet weight) of the whole body composition of *P. vachelli* (darkbarbel catfish) fed the diets with graded levels of SPC

Diet abbreviation	*Whole-body composition (%)*		
	Crude protein	*Crude lipid*	*Moisture*
SPC100%	14.8±0.14	13.4±0.02	69.3±0.003
SPC80%	14.6±0.14	12.6±0.02	70.0±0.003
SPC60%	14.2±0.16	12.2±0.01	70.8±0.004
SPC40%	15.1±0.14	12.8 ±0.01	69.1±0.008
SPC20%	15.2±0.22	13.4±0.01	68.6±0.007
SPC0%	14.0±0.11	12.7±0.01	71.0±0.025

Data represent mean ± SEM (n = 3).
Values in the same column with the same superscript letters are not significantly different ($P > 0.05$).

Apparent digestibility analyses. The ADC of dry matter decreased with increasing dietary SPC ($P < 0.05$) (Table 5). However, the ADC of dry matter in fish fed the SPC 20% diet showed no significant difference compared with the control diet ($P > 0.05$). Similarly, the ADC of crude protein and crude lipid decreased with increasing dietary SPC ($P < 0.05$). No significant differences were observed among fish fed the diets SPC 0, 20, 40 and 60%.

Table 5. Apparent digestibility coefficients of nutrients of *P. vachelli* (darkbarbel catfish) fed diets with graded levels of SPC

Diet abbreviation	*Apparent digestibility coefficients (%)*		
	ADC of dry matter	*ADC of crude protein*	*ADC of crude lipid*
SPC100%	61.63±0.36^{a}	84.38±0.83^{a}	80.46±0.22^{a}
SPC80%	65.69±0.33^{b}	85.19±0.16^{a}	82.28±0.23^{a}
SPC60%	68.88±0.63^{bc}	87.86±0.15^{ab}	84.22±1.00^{abc}
SPC40%	71.40±1.06^{c}	88.02±0.33^{ab}	85.47±0.86^{bc}
SPC20%	77.11±1.24^{d}	88.96±0.54^{b}	86.09±1.00^{bc}
SPC0%	75.48±0.46^{d}	88.89±0.17^{b}	86.95±0.35^{c}

ADC: Apparent digestibility coefficients.
Data represent mean ± SEM (n = 3).
Values in the same column with the same superscript letters are not significantly different (P > 0.05).

Protein digestive enzymes assay. No significant differences were observed in pepsin levels among dietary treatments (P > 0.05) although it exhibited a decreasing trend with increasing dietary SPC levels (Table 6). Trypsin activity had a maximum value of 60.26 U/mg protein at 20% substitution level and decreased with increasing dietary SPC levels (Table 6) (P < 0.05). However, trypsin activity in fish fed the diets SPC 20, 40 and 60% was not significantly different from the control diet.

Table 6. Digestive enzymes activity, protein digestive enzyme and metabolism enzymes of *P. vachelli* (darkbarbel catfish) fed the diets with graded levels of SPC

Diet abbreviation	*Enzyme / Tissue (U mg^{-1} protein)*			
	Pepsin/Stomach	*Trypsin/ Gut*	*ALT/ Liver*	*AST/ Liver*
SPC100%	24.69±1.57^{a}	42.25±0.34^{a}	55.56±2.12^{a}	57.54±2.44^{a}
SPC80%	25.63±3.56^{a}	50.41±0.57^{b}	77.21±1.57^{b}	64.19±0.96^{b}
SPC60%	25.55±1.23^{a}	56.69±0.44^{c}	99.25±1.02^{c}	68.98±1.71^{ab}
SPC40%	30.83±4.19^{a}	57.75±0.54^{c}	104.17±1.87^{c}	70.94±2.03^{abc}
SPC20%	32.75±5.30^{a}	60.26±0.58^{d}	116.73±2.85^{d}	84.16±1.36^{bc}
SPC0%	29.33±3.33^{a}	58.70±0.29^{cd}	107.52±2.50^{cd}	85.01±2.09^{c}

Data represent mean ± SEM (n = 3).
Values in the same column with the same superscript letters are not significantly different (P > 0.05).

Protein metabolism enzymes assay. ALT activity in liver decreased with increasing dietary SPC levels (P < 0.05) (Table 6). No significant differences in ALT activity were observed in fish fed the diets with 60% or less SPC compared to the control diet. AST activity in liver also showed the same trend as liver ALT activity. However, fish fed the diets with 40% SPC or less showed no significant differences compared with the control diet.

Intestine histology. As dietary SPC increased from 0-100%, significant changes were observed in the histology of the distal intestine. These exhibited reduced mucosal fold height, loss of normal enterocyte supranuclear absorptive vacuolization, widening of the central stroma within the mucosal folds, and increased amounts of connective tissue (Fig.1).

Cost benefit analysis. Among the formulated diets, the diet with SPC 60% had the highest profit index, which meant it cost less to produce one kilogram of fish biomass (Table 7) (P < 0.05). However, no significant differences in PI were observed in fish fed the diets SPC 20%, 40%, 60% and 80%.

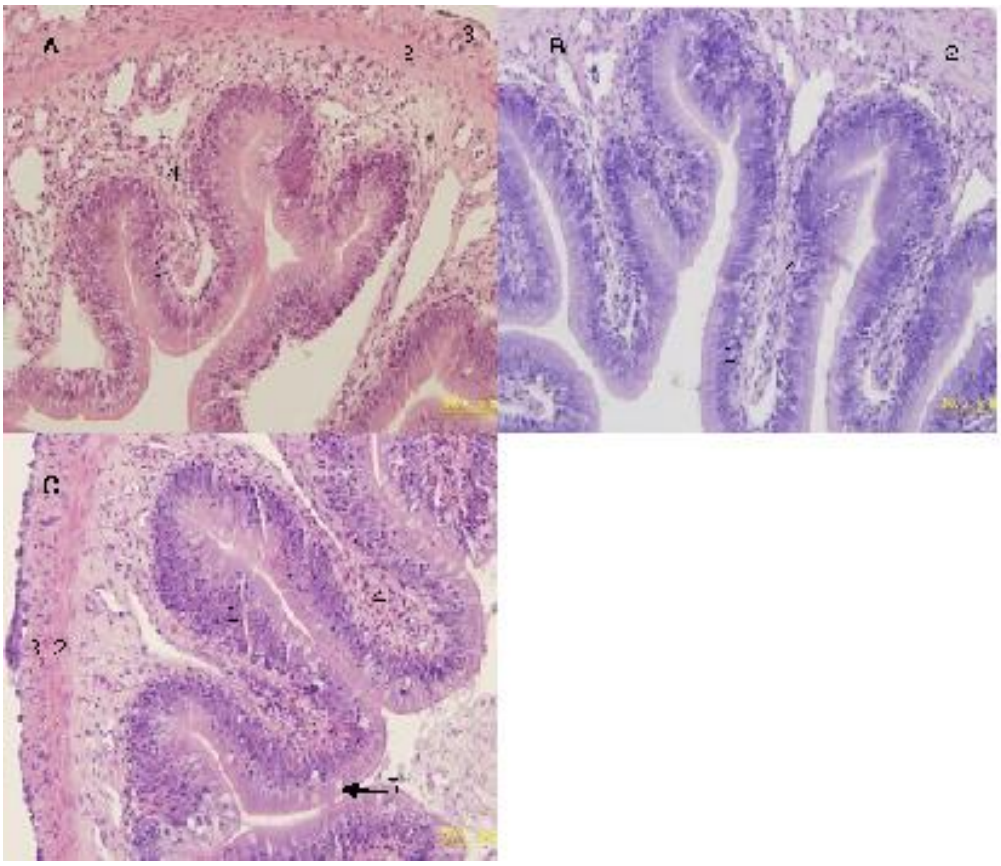

Fig.1. Cross sections of the distal intestine of *P. vachelli* (darkbarbel catfish) fed with different levels of SPC (SPC 100%, SPC 40%, SPC 0%) (A, B, C).
Fish fed the SPC diet exhibit an overall shortening and fusion of mucosal folds, reduced to absent absorptive vacuolization, and hyperplastic lamina propria with concomitant leukocyte infiltration of the lamina propria and submucosa. (1) epithelium (2) stratum compactum (3) stratum granulosum (4) Lamina propria (5) goblet cells. Scale bars=50 µm.

Table 7. Cost analysis data for *P. vachelli* (darkbarbel catfish) fed diets with graded levels of SPC.

Diet abbreviation	*Parameter*				
	TFC(g)	*FC(US $)*	*TNBG(g)*	*PG(US $)*	*PI*
SPC100%	466.67^{a}	1.27^{a}	364.15^{a}	2.18^{a}	1.72^{a}
SPC80%	639.34^{ab}	1.42^{ab}	519.33^{b}	3.12^{b}	2.20^{bc}
SPC60%	719.46^{bc}	1.56^{ab}	587.48^{bc}	3.52^{bc}	2.25^{c}
SPC40%	760.60^{bc}	1.71^{bc}	636.95^{c}	3.82^{bc}	2.24^{c}
SPC20%	794.48^{bc}	1.85^{bc}	661.31^{cd}	3.97^{bc}	2.14^{bc}
SPC0%	807.66^{c}	2.00^{c}	678.66^{d}	4.07^{c}	2.03^{b}

TFC: Total feed consumed; FC: Feed cost; TNBG: Total net biomass gain; PG: Profit gain; PI: Profit index.
Data represent mean ± SEM (n = 3).
Values in the same column with the same superscript letters are not significantly different (P > 0.05).

Discussion

In the present study, growth was significantly affected by dietary SPC. With increasing dietary SPC, growth significantly decreased. SGR was significantly lower in fish fed the diets with more than 80% of the FM protein replaced bySPC, than in the control group. Meanwhile, no significant differences in SGR were observed in fish fed the diets with 60% or less SPC compared with the control diet. These results suggested that 60% of FM protein could be replaced by SPC without significantly affecting growth, and higher

substitution levels resulted in growth reduction. Similar results were also reported in previous publications on rainbow trout (Mambrini et al. 1999), Senegalese sole (Aragão et al. 2003), Japanese flounder (Deng et al. 2006), yellowtail (Takagi et al. 2006), rainbow trout (Collins et al.2012) and juvenile yellowtail kingfish (Bowyer et al. 2013). However, studies on rainbow trout (Kaushik et al. 1995) and halibut (Berge et al. 1999) reported that substitution of FM with SPC at rates up to 80% or 100% in feeds showed no adverse effects on growth performance of these species. These differences could be due to different species, age, dietary composition, feeding strategy, and other factors.

The reduction in diet palatability usually results in a decrease in FI, which could in turn cause reduced growth (Aragão et al. 2003). In this study, FI decreased with the increase of dietary SPC. This is consistent with findings in other studies on rainbow trout (Dias et al.1999), juvenile Japanese flounder (Deng et al. 2006) and juvenile yellowtail kingfish (Bowyer et al. 2013). This was attributed to methionine deficiency in the SPC based diet. EAA mixture supplementation in diets with 75% SPC replacement levels significantly increased FI of juvenile Japanese flounder compared to FI fed the diet without EAA mixture supplementation (Deng et al. 2006). SPC may have certain levels of phytic acid and bitter off-flavor (Storebakken et al. 2000a; Bowyer et al. 2013), although most anti nutritional factors (ANF) were removed or deactivated, however this could affect flavor, reduce palatability of the diets and diminish the appetite of the fish. Hence, suppression of FI could be the main reason for reduced growth performance of juvenile *P. vachelli*.

The reduced growth may also be due to lower nutrient digestibility. In the present study, the ADC of nutrients, especially protein, with incorporation of 80% SPC or more were significantly lower than those in the control diet. The results presented in Table 5 demonstrate that the ADC of nutrients decreased with increasing dietary SPC levels. It can be concluded that digestibility is an important limiting factor when SPC was used as the protein source for juvenile *P. vachelli*. The ADC of protein declined with the increase of dietary SPC in Japanese flounder juvenile (Deng et al. 2006).

The SPC diet could result in extensive pathologies of digestive tract and most likely affect nutrient utilization in rainbow trout and pacu (Teresa et al. 2005). Research on rainbow trout has shown that SPC did not cause inflammatory reaction of the gut nor did it affect the epithelium surface (Escaffre et al. 2007). However, in the present study, some DI morphological changes were observed with increasing dietary SPC. The DI histological changes of fish fed 100% SPC were as pronounced as the fully developed soy induced enteritis (Van den Ingh et al. 1991; Baeverfjord and Krogdahl 1996; Bakke et al. 2000). The most likely explanation for this result was that the quality of SPC extraction was inadequate. Similar results were also observed in some studies with rainbow trout (Nordrum et al. 2000; Refstie et al. 2000) and Atlantic salmon (Krogdahl et al. 2003). Dietary SPC levels could affect nutrient digestibility if the SPC damaged the DI epithelium integrity, thus depressing the pepsin and trypsin enzyme activity, finally decreasing nutrient digestibility.

ALT and AST activity in the liver declined with increasing dietary SPC, which indicated that protein utilization was reduced and the liver was damaged to some extent. Aminotransferases, such as ALT and AST, catabolize amino acids and transfer amino groups to alpha-keto acids (reversible catalysis). When the available essential amino acids are deficient, the keto acids may be diminished, thereby reducing ALT and AST activity (Cheng et al. 2010). In the present study, increasing dietary SPC, decreased dietary methionine levels. Since this is usually the first limiting amino acid in many fish diets fish growth was significantly affected. (Mai et al. 2006; Zhou et al. 2006). Hence, the decline of ALT and AST activity in the liver could be associated with the imbalance of essential amino acids and deficiency of methionine, which also accounted for reduced growth at high substitution levels of SPC.

Conclusion

The present experiment demonstrated that high dietary SPC levels (80% or higher) resulted in significant adverse effects on growth performance, nutrient digestibility, protein metabolism and gut integrity of juvenile *P. vachelli*. Fish meal protein could be substituted with up to 60% SPC protein in diets of catfish under the experimental conditions described.

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