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Effects of the partial substitution of dietary fish meal by two types of soybean meals on the growth performance of juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier 1828)

Yan Li, Qinghui Ai, Kangsen Mai, Wei Xu & Zhenyan Cheng

The key laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao, China

Correspondence: Q H Ai, The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao 266003, China. E-mail: qhai@ouc.edu.cn

Abstract

The effect of partial substitution of dietary fish meal with a high-value soybean meal (HVS) and a commercial soybean meal (CSM) on the growth performance in juvenile Japanese seabass (8.3 ± 0.2 g body weight) was determined. Nine isonitrogenous (crude protein 44%) and isoenergetic (20 kJ g^{-1}) practical diets replacing 0 (the control), 15%, 30%, 45% and 60% fish meal protein by soybean meal protein (HVS or CSM) were formulated. Each diet was randomly assigned to four replicate cages (1.5 × 1.5 × 2.0 m). Fish were fed twice daily for 10 weeks. Growth was significantly reduced with increased soybean inclusion in fish fed both the soybean sources. Independent of the soybean source used, increased inclusion of soybean meal decreased the growth performance. When the substitution level was 45% or more in CSM and 60% in HVS, the specific growth rate (SGR) and the feed efficiency ratio (FER) were significantly lower compared with the control. These results indicated that CSM protein could substitute for 30% fish meal protein, while the substitution level of HVS protein could be 45%, which did not influence the growth of juvenile Japanese seabass. The higher substitution level for HVS compared with CSM was probably due to better nutritional values.

Keywords: high value soybean meal, commercial soybean meal, replacement, fish meal, juvenile Japanese seabass, growth

Introduction

Reducing the cost in aquaculture feeding has long been a main focus for fish nutritionists. One approach is to replace the increasingly expensive fish meal, a major protein source in aquatic feeds, with less expensive and readily available plant proteins. Of all plant proteins, soybean meal is the most promising alternative protein source because of its high protein content and relatively balanced amino acid profile (Refstie, Storebakken, Baeverfjord & Roem 2001; Ai & Xie 2005). Some previous studies have shown that soybean meal was able to partially or completely replace fish meal without compromising the growth and feed utilization in fish. However, the potential of replacement depends on the type of soybean meal due to the differences in the protein level, amino acid profiles, the levels of antinutritional factors and the digestibility of nutrients and energy (Kaushik, Cravedi, Lallès, Sumpter, Fauconneau & Laroche 1995; Refstie *et al.* 2001). Recently, advances in plant biotechnology and breeding have led to the development of high-value soybean (HVS) in the United States. The meal generated from this HVS contains up to 60% crude protein, higher than that of the normal commercial soybean meal (CSM). However, the potential of HVS meal has not been investigated in aquafeeds (Liang, Kotowski & Chi 2005).

Japanese seabass is a carnivorous species that has been cultured widely in China because of its delicious meat and rapid growth. It has become an important and the largest cultured scale species in China. To date, a few preliminary studies have been conducted on its nutrient requirements (Ai, Mai, Li, Zhang, Zhang, Duan, Tan, Xu, Ma, Zhang & Liufu 2004; Ai, Mai, Zhang, Xu, Duan, Tan & Liufu 2004; Mai, Zhang, Ai, Duan, Zhang, Li, Wan & Liufu 2006; Zhang, Mai, Ai, Zhang, Duan, Tan, Ma, Xu, Liufu &

Wang 2006). However, the potential of replacing fish meal by HVS meals has not been investigated. The present study was designed to compare the effects of partial replacement of fish meal by HVS and a normal CSM on the growth performance and body composition of this juvenile seabass. The results may also apply to closely related farmed fish species.

Materials and methods

Experimental diets

De-hulled HVS (obtained from RENESSEN, Bannockburn, IL, USA) with 61% (dry weight) crude protein and the regular commercial de-hulled soybean meal (CSM, obtained from commercial market) with 53% (dry weight) crude protein were used to replace fish meal in a feeding experiment. Nine isonitrogenous (crude protein 44%) and isoenergetic ($20\,kJ\,g^{-1}$) practical diets replacing 0 (the control), 15%, 30%, 45% and 60% fish meal protein by soybean meal protein (HVS or CSM) were formulated (Table 1 for CSM and Table 2 for HVS). Encapsulated amino acids with tripalmitin were supplemented to meet the essential amino acid requirements based on the whole-body amino acid composition of Japanese seabass (Tables 3 and 4). $400\,mg\,kg^{-1}$ yttrium oxide (Y_2O_3, Fluka Chemicals®, Castle Hill, NSW, Australia) was used as an inert marker in each diet to determine the apparent digestibility. Ingredients were ground into a fine powder through a 320 μm mesh. All the ingredients were thoroughly mixed with menhaden fish oil, and water was added to produce a stiff dough. The dough was then pelleted using an experimental feed mill [F-26 (II), South China University of Technology, Guangzhou, China] and dried for about 12 h in a ventilated oven at 60 °C. After drying, the diets were broken up and sieved into proper pellet sizes (1.5 × 3.0 and 2.5 × 5.0 mm), and were stored at −20 °C until used.

Experimental procedure of the feeding trial

Japanese seabass was obtained from Ningbo Fisheries Corporation, Zhejiang, China. Before the start of the experiment, the juvenile seabass was reared for 10 days to acclimate to the experimental diets and conditions.

At the start of the experiment, the fish were fasted for 24 h and weighed after being anaesthetized with eugenol (1:10 000) (Shanghai Reagent Corporation, Shanghai, China). Fish of similar sizes (8.3 ± 0.2 g) were randomly distributed into 36 seawater floating cages (1.5 × 1.5 × 2.0 m), and each cage was stocked with 30 fish. Each diet was randomly assigned to four replicate tanks. Fish was hand-fed to apparent satiation twice (06:30 and 16:30 hours) daily for 70 days. During the experimental period, the temperature was about 18.0–24.5 °C, the salinity was 26–30 $mg\,L^{-1}$ and the dissolved oxygen content was approximately $7\,mg\,L^{-1}$. At the termination of the experiment, the fish were fasted for 24 h before harvest. The total number and mean body weight of fish in each cage were measured.

Digestibility test

The apparent digestibilities of HVS and CSM used in the growth experiment were determined. The control diet in the growth experiment was used as a reference diet. The reference diet was mixed with test ingredients and yttrium oxide (Shanghai Reagent Corporation) in a 69.9:30:0.1 ratio to produce test diets (Chou, Slinger & Bayley 1982). The reference and test diets were fed to Japanese seabass (initial weight 8.3 ± 0.15 g) reared under the same conditions as that of the growth experiment.

After a 5-week feeding, the faecal sample for five fish in each cage was collected by stripping and the apparent digestibility was measured. The daily samples for each tank were combined into a single sample and frozen at approximately −20 °C until analysis.

Analysis and measurement

Chemical analysis of diets and fish body composition

Proximate composition analyses on the experimental diets and fish body were performed using the standard methods of Association of Official Analytical Chemists (1995). Samples of diets and fish were dried to a constant weight at 105 °C to determine moisture. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method and lipid by ether extraction using Soxhlet. Phosphorus in the diets was analysed using an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, VARIAN, Palo Alto, CA, USA) after perchloric acid digestion. For amino acids (except for methionine and cystine), the feed ingredients was freeze-dried, and then hydrolysed with 6 N HCl at 110 °C for 22 h and analysed using a Biochrom 30 amino acid analyser (Biochrom®, Cambridge, UK).

Table 1 Formulation and proximate composition of experimental diets containing a commercial soybean meal (% dry matter)

Ingredient	Diet no. (substitution level) Diet 1 (0%)	Diet 2 (15%)	Diet 3 (30%)	Diet 4 (45%)	Diet 5 (60%)
Fish meal*	52.0	44.2	36.4	28.6	20.8
Commercial soybean meal*	0.0	10.5	21.0	31.5	42.0
Beer yeast*	4.0	4.0	4.0	4.0	4.0
Menhaden fish oil	1.0	2.2	3.5	4.8	6.0
Soybean oil	1.0	0.9	0.8	0.7	0.6
Wheat meal	26.0	24.0	23.0	21.0	18.0
Vitamin premix†	2.0	2.0	2.0	2.0	2.0
Mineral premix‡	2.0	2.0	2.0	2.0	2.0
Starch§	5.00	3.80	1.90	0.50	0.08
Attractant¶	0.5	0.5	0.5	0.5	0.5
Lecithin	2.0	2.0	2.0	2.0	2.0
Antioxidant ‖	0.05	0.05	0.05	0.05	0.05
Yttrium oxide	0.04	0.04	0.04	0.04	0.04
Amino acid premix	0.42	0.53	0.65	0.82	1.00
Cellulose**	3.99	2.78	1.46	0.69	0.03
Proximate composition					
Dry matter (%)	91.3	91.2	91.5	91.2	91.0
Crude protein (%)	44.4	44. 5	44.2	44.0	43.8
Crude lipid (%)	11.2	11.8	11.6	11.5	11.8
Digestible phosphorus (%)	0.93	0.82	0.72	0.61	0.57

*Fish meal, white fish meal, obtained from Hangzhou Wensli Biology Science and Technology Corporation (Zhejiang, China), crude protein, 71.0% dry matter, crude lipid, 16.2% dry matter; soybean meal, obtained from commercial market, crude protein, 52.7% dry matter, crude lipid, 1.3% dry matter; wheat meal, crude protein, 16.2% dry matter, crude lipid, 2.0% dry matter; beer yeast, crude protein, 54.5% dry matter, crude lipid, 2.5% dry matter.

†Vitamin premix (mg or g kg diet^{-1}), 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; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2000 mg, ethoxyquin, 150 mg, wheat middling, 14.52 g.

‡Mineral premix (mg or g kg diet^{-1}), 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; $MgSO_4 \cdot 7H_2O$, 1200 mg; Ca $(H_2PO_4)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; Zoelite, 15.448 g.

§Starch: Corn starch.

¶Attractant: Dimethyl-β-propiothetin-DMPT.

‖Antioxidant: Ethoxyquin, obtained from Qingdao Master Biotechnology, Qingdao, China.

**Cellulose: Microcrystalline cellulose.

Calculations

The following variables were calculated:

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100/t$

Feeding rate (FR) = $100 \times I \times 2/((W_t + W_0) \times t)$

Survival rate (%) = $100 \times (N_t/N_0)$

Feed efficiency ratio (FER) = Wet weight gain in g/ dry feed fed in g

Apparent digestibility coefficient (ADC) of dry matter (%) = (1 − dietary Y_2O_3/faecal Y_2O_3) × 100

Apparent digestibility coefficient of nutrients or energy (%) = (1 − (dietary Y_2O_3/faecal Y_2O_3) × (faecal nutrient or energy/dietary nutrient or energy)) × 100

Apparent digestibility coefficient of the test ingredient (%) = 100/30 × (ADC in the test diet − 0.6996 × ADC in the reference diet) (Chou *et al.* 1982) where W_t and W_0 are the final and the initial body weight respectively; t is the duration of the experimental days; N_t and N_0 are the final and the initial number of fish respectively; and I (g) is feed intake as dry matter.

Statistical analysis

All data were analysed using analysis of variance and regression analysis where appropriate using SPSS 12.0 for windows. Data were analysed using one- and two-way analysis of variance to determine whether there were significant differences ($P < 0.05$) due to the dietary levels of substitution, soybean sources or the interaction. t-test (variance ratio) was also used to determine the significant difference in antinutritional factors.

Table 2 Formulation and proximate composition of experiment diets containing Renessen HVS meal (% dry matter)

Ingredient	Diet no. (substitution level)				
	Diet 1 (0%)	Diet 6 (15%)	Diet 7 (30%)	Diet 8 (45%)	Diet 9 (60%)
Fish meal*	52.0	44.2	36.4	28.6	20.8
Renessen soybean meal*	0.0	9.1	18.3	27.4	36.6
Beer yeast*	4.0	4.0	4.0	4.0	4.0
Menhaden fish oil	1.0	2.2	3.5	4.7	6.0
Soybean oil	1.0	0.9	0.8	0.7	0.6
Wheat meal	26.0	23.5	21.0	19.0	17.0
Vitamin premix†	2.0	2.0	2.0	2.0	2.0
Mineral premix‡	2.0	2.0	2.0	2.0	2.0
Starch§	5.0	4.9	4.8	4.6	4.3
Attractant¶	0.5	0.5	0.5	0.5	0.5
Lecithin	2.0	2.0	2.0	2.0	2.0
Antioxidant ‖	0.05	0.05	0.05	0.05	0.05
Yttrium oxide	0.04	0.04	0.04	0.04	0.04
Amino acid premix	0.42	0.64	0.86	1.10	1.37
Cellulose**	3.99	3.57	3.15	2.51	1.84
Proximate composition					
Dry matter (%)	91.3	91.2	92.3	91.0	91.0
Crude protein (%)	44.4	44.0	44.3	44.1	44.6
Crude lipid (%)	11.2	11.1	11.2	11.7	11.9
Digestible phosphorus (%)	0.93	0.90	0.83	0.71	0.64

*Fish meal, white fish meal, obtained from Hangzhou Wensli Biology Science and Technology Corporation (Zhejiang, China), crude protein, 71.0% dry matter, crude lipid, 16.2% dry matter; soybean meal, obtained from Renessen,IL, USA, by cracking, heating and flaking soybeans to make solvent-extracted dehulled soybean meal, crude protein, 60.6% dry matter, crude lipid, 1.7% dry matter; wheat meal, crude protein, 16.2% dry matter, crude lipid, 2.0% dry matter; beer yeast, crude protein, 54.5% dry matter, crude lipid, 2.5% dry matter.
†Vitamin premix (mg or g kg diet $^{-1}$), 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; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2000 mg, ethoxyquin, 150 mg, wheat middling, 14.52 g.
‡Mineral premix (mg or g kg diet $^{-1}$), 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; $MgSO_4 \cdot 7H_2O$, 1200 mg; Ca $(H_2PO_4)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; Zoelite, 15.448 g.
§Starch: Corn starch.
¶Attractant: Dimethyl-β-propiothetin-DMPT.
‖Antioxidant: Ethoxyquin, obtained from Qingdao Master Biotechnology.
**Cellulose: Microcrystalline cellulose.
HVS, high-value soybean meal.

Result

Survival and growth performance

The survival rate ranged from 90.8% to 95.8% in the present study, and was independent of the dietary soybean protein level in both soybean proteins (Table 5). At the conclusion of the 10-week trial, growth was significantly affected by dietary different soybean sources and supplementation levels. The SGR showed decreasing trends with increasing dietary soybean protein, and SGR of juvenile fish fed HVS grew better than those of CSM at the same substitution level. The interaction of dietary soybean species and inclusion levels also significantly affected the growth of fish. Specific growth rate of fish fed the highest dietary HVS protein (60%) and 45%, 60% protein from CSM were significantly lower than the other groups. Feed efficiency rate and FR were significantly decreased with increasing dietary soybean protein. However, there was no interaction of dietary soybean protein and soybean species on FER or FR.

The regression equation of HVS and SGR is SGR = − 0.0059 HVS + 3.6773 ($P < 0.001$), and SGR = − 0.0115 CSM + 3.7102 ($P < 0.001$) for CSM. It can be concluded that SGR decreased with increasing dietary soybean protein in both HVS and CSM. However, with increasing dietary soybean protein, SGR in CSM decreased more quickly compared with HVS because of a lower slope (Fig. 1).

The SGR of Japanese seabass were positively correlated with dietary available phosphorus in both soybean proteins, and the correlations were significant ($P < 0.05$) (Fig. 2).

Table 3 Nutritional values between HVS and normal CSM

Soybean meal	HVS	CSM
Protein (g kg^{-1} dry weight)	606	527
Lipid (g kg^{-1} dry weight)	17	13
Moisture (g kg^{-1})	111	101
Amino acids (g kg^{-1} wet weight)		
Asp	58.3	54.8
Thr	19.9	19.8
Val	33.9	34.3
Met	6.3	6.4
Ile	23.6	22.8
Leu	38.0	36.6
Phe	24.2	23.1
Lys	31.2	30.3
His	13.5	12.9
Arg	39.9	34.8

HVS, high-value soybean meal; CSM, commercial soybean meal.

Table 4 Composition of amino acid premix in Diet 1–Diet 9 (% dry matter)

Diet no.	Arg	Lys	Met	Leu	Total
Diet 1	0.1	0.27	0.04	–	0.42
Diet 2	0.04	0.35	0.13	–	0.53
Diet 3	–	0.43	0.22	–	0.65
Diet 4	–	0.5	0.32	–	0.82
Diet 5	–	0.59	0.41	–	1.00
Diet 6	0.1	0.4	0.15	–	0.64
Diet 7	0.09	0.53	0.25	–	0.86
Diet 8	0.08	0.65	0.35	0.02	1.10
Diet 9	0.06	0.77	0.45	0.08	1.37

Therefore, 30% protein could be replaced by CSM and 45% by HVS in the diets of juvenile Japanese seabass.

Whole-body composition

No significant differences were observed in body protein (16.3–17.0%), lipid (5.8–7.7%) and moisture (70.8–72.4%) among dietary treatments ($P > 0.05$) (Table 6).

The body compositions were not affected by dietary soybean sources, inclusion levels or the interaction of these two factors.

Nutrient digestibility of two soybean meals and diets

Apparent digestibility coefficients of dry matter and crude lipid in HVS meal were higher than those in CSM, but no significant differences were observed ($P > 0.05$) (Table 7). However, ADC of crude protein in HVS was significantly higher than that of CSM ($P < 0.05$).

Apparent digestibility coefficients of nutrients showed a decreasing trend with increasing dietary soybean protein levels in both soybean sources (Table 8). When the substitution level was 450 g kg^{-1} or more in CSM, and 600 g kg^{-1} in HVS, ADCs of dry matter, crude protein and lipid were significantly lower compared with the control ($P < 0.05$). No significant difference was observed in ADC of phosphorus in HVS compared with the control; however, ADC of phosphorus was significantly lower than the control when the substitution level was 600 g kg^{-1} in CSM. Apparent digestibility coefficients of dry matter, crude protein, lipid and phosphorus in HVS were relatively higher than those in CSM at the same substitution level. The interaction of dietary soybean species and inclusion levels did not significantly affect ADCs of dry matter, crude protein, lipid and phosphorus.

Discussion

The growth of juvenile Japanese seabass was significantly affected by dietary soybean protein levels in both soybean proteins. With increasing dietary soybean protein, growth decreased significantly. When the substitution level was 45% or more in CSM and 60% in HVS, the SGR were significantly lower compared with the control. These results indicated that CSM protein could substitute 30% fish meal protein, while the substitution level of HVS meal protein could achieve 45%, which did not influence the growth of juvenile Japanese seabass. On regression analysis, SGR in CSM decreased quicker compared with HVS with increasing dietary soybean protein, and the growth of juvenile Japanese seabass fed the diets containing HVS were better than those of CSM at the same substitution levels, suggesting that HVS was better utilized by juvenile Japanese seabass.

Some previous studies showed that the FR decreased with increasing dietary soybean protein, which accounted for growth reduction (Hajen, Higgs, Beames & Dosanjh 1993; Tantikitti, Sangpong & Chiavareesajja 2005; Deng, Mai, Ai, Zhang, Wang, Xu & Liufu 2006). In the present study, however, the FR did not decrease with increasing dietary soybean protein, and when the substitution level increased, the FR increased significantly. This result was paral-

Table 5 Growth performances of juvenile Japanese seabass* fed diets with graded levels of soybean proteins from CSM or HVS meal†

Diets no. (substitution level)	Final weight (g)	Specific growth rate (SGR) (% day^{-1})	Feed efficiency rate (FER)	Feeding rate (FR) (% 100 g^{-1} day^{-1})	Survival (%)
Diet 1 (0)	105.4[a]	3.63[a]	0.96[a]	2.55[d]	95.8
Diet 2 (15%)	101.0[ab]	3.57[a]	0.93[a]	2.61[cd]	94.2
Diet 3 (30%)	95.6[b]	3.49[a]	0.90[a]	2.67[bcd]	94.2
Diet 4 (45%)	76.3[c]	3.17[b]	0.76[c]	3.01[b]	90.8
Diet 5 (60%)	66.4[d]	2.97[c]	0.60[d]	3.71[a]	92.5
Diet 6 (15%)	102.6[ab]	3.59[a]	0.94[a]	2.59[cd]	94.2
Diet 7 (30%)	100.1[ab]	3.56[a]	0.88[ab]	2.76[bcd]	95.0
Diet 8 (45%)	94.9[b]	3.48[a]	0.81[bc]	2.97[bc]	93.3
Diet 9 (60%)	80.5[c]	3.24[b]	0.67[d]	3.51[a]	93.3
Pooled SEM	2.25	0.04	0.02	0.07	0.58
Two-way ANOVA			*F* value		
Substitution level	87.340	91.712	106.018	49.549	1.134
Soybean sources	49.446	56.712	3.598	0.546	0.670
Level × sources	8.517	10.852	2.460	1.057	0.170
			P value		
Substitution level	0.000	0.000	0.000	0.000	0.361
Soybean sources	0.000	0.000	0.069	0.466	0.420
Level × sources	0.000	0.000	0.084	0.384	0.916

*The initial weight of juvenile Japanese seabass was 8.3 ± 0.2 g BW.
†Values are presented as means with pooled SEM. Values in the same column with the same superscripts are not significantly different as determined using Tukey' test ($P > 0.05$).
CSM, commercial soybean meal; HSV, high-value soybean meal (Renessen).

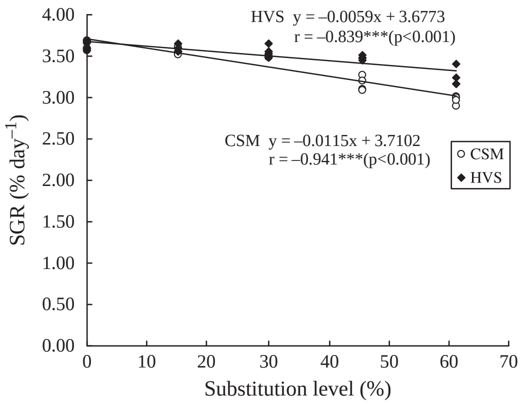

Figure 1 Regression between the two soybean proteins substitution levels (*x*) and specific growth rate (SGR) (*y*) of Japanese seabass. Note: treatments marked (***) are significantly different from the control group at $^{***}P < 0.001$.

leled by the findings in rainbow trout and Atlantic salmon (Refstie, Helland & Storebakken 1997; Refstie, Storebakken & Roem 1998), which indicated that the feeding rate could be readily accepted by juvenile Japanese seabass. Hence, it was not the reason for growth depression induced by a high level of soybean meal substitution in the present study.

Soybean meals contain many antinutritional factors that have been considered to be major factors responsible for impaired growth performance. The contents of antinutritional factors such as phytic acid are major impediments in efforts towards increased use of vegetable feed ingredients in diets for fish (Storebakken, Refstie & Ruyter 2000). Phosphorus deficiency and reduction in phosphorus bioavailability due to phtytate in SBM could reduce the growth of many fish species (National Research Council 1993; Chou, Her, Su, Hwang, Wu & Chen 2004; Lim, Choi, Wang, Kim, Shin, Min & Bai 2004; Liebert & Portz 2005). This study found that there was a significant correlation between dietary available phosphorus and SGR of juvenile Japanese seabass, and the SGR increased with increasing available phosphorus. When the substitution level was 45% or more in CSM and 60% in HVS, SGR were significantly lower compared with the control. Especially, the growth of fish fed the diets containing relatively higher available phosphorus of HVS was better than those of CSM at the same substitution levels. This was in agreement with Zhang *et al*. (2006), who found that the growth response was significantly affected by the dietary available phosphorus. In addition, many studies showed that as the dietary soybean meal inclusion increased, methionine was an important limitation (Wilson & Poe 1985; Olli, Hjelmeland & Krogdahl 1994; Ai & Xie 2005). In this study,

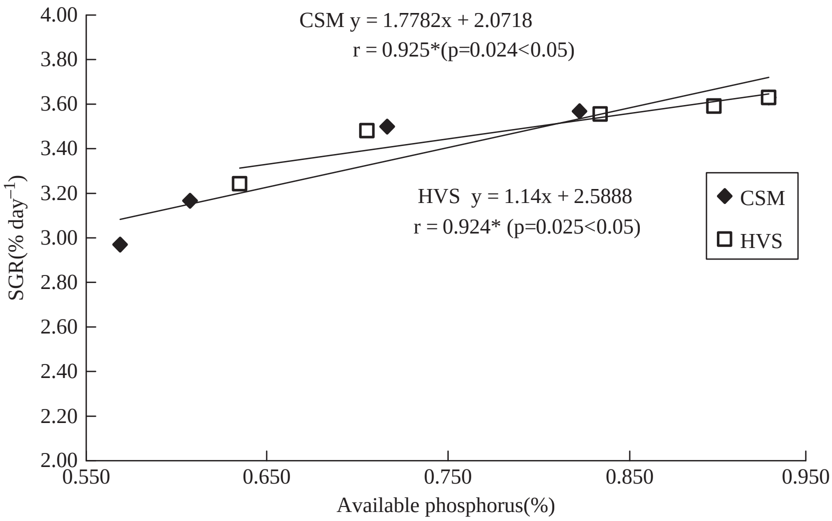

Figure 2 Regression between digestible phosphorus in diets (x) and specific growth rate (SGR) (y) of Japanese seabass. Note: treatments marked (*) are significantly different from the control group at $^*P<0.05$.

Table 6 Proximate composition (% wet weight) of the whole body of fish fed diets with graded levels of soybean proteins from CSM or HVS meal*

Diets no. (substitution level)	Moisture (%)	Crude protein (%)	Crude lipid (%)
Diet 1 (0)	72.1	16.6	6.5
Diet 2 (15%)	71.9	16.8	5.8
Diet 3 (30%)	71.5	16.3	7.7
Diet 4 (45%)	71.1	16.7	6.6
Diet 5 (60%)	72.4	16.6	6.0
Diet 6 (15%)	70.8	17.0	6.7
Diet 7 (30%)	71.4	16.6	7.1
Diet 8 (45%)	71.7	16.7	6.8
Diet 9 (60%)	72.0	16.4	6.7
Pooled SEM.	0.2	0.1	0.2
Two-way ANOVA		*F* value	
Substitution level	0.550	0.600	0.600
Soybean sources	0.974	1.421	1.421
Level × sources	0.847	0.769	0.769
		P value	
Substitution level	0.467	0.805	0.442
Soybean sources	0.424	0.490	0.239
Level × sources	0.484	0.804	0.516

*Values are presented as means with pooled SEM. Values in the same column with the same superscripts are not significantly different as determined using Tukey' test ($P>0.05$).

CSM, commercial soybean meal; HVS, high-value soybean meal.

methionine was supplemented to meet the essential amino acid requirements based on the whole-body amino acid composition of juvenile Japanese seabass. Therefore, methionine was not an important limitation factor in this study. From the above conclusion, higher dietary antinutritional factors such as phytate and deficiency of available phosphorus were the two parameters that explain the reduced growth due to the different soybean sources and inclusion levels.

It is generally accepted that the inefficiency of supplementation with crystalline amino acids (CAA) was due to the faster uptake and subsequent catabolism of the supplemented amino acids compared with those from intact protein (Murai, Ogata, Hirasawa, Akiyama & Nose 1987; Cowey & Walton 1988), and the leaching loss of CAA in aquatic environments (Zarate & Lovell 1997). In this study, the encapsulated amino acids with tripalmitin were supplemented to delay the rate of absorption of free amino acids, and to prevent amino acid loss due to sea current, fish were hand-fed slowly till apparent satiation (visual observation of fish feeding behaviour). However, the results of the present study showed that supplementation of crystal essential amino acids could not improve the growth of experimental fish, suggesting that crystal amino acids cannot be efficiently utilized by this seabass. Furthermore, the lower utilization efficiency of coating materials in this study may be one of the limiting factors for the growth of seabass. Kaushik, Cove's, Dutto and Blanc (2004) first demonstrated an almost total replacement of fish meal and soybean meal by a mixture of other plant protein sources in European seabass, which suggested that using the soybean meal as one protein substitution may not be better than several plant proteins together, even though supplemented the coating amino acids which cannot be used effectively by fish. According to the amino acid analysis in both kinds of soybean meals, HVS contained relatively more amino acids than those in CSM, which can also, to some extent, explain

Table 7 Apparent digestibility coefficients (%) of dry matter, crude protein and lipid in two soybean meals*

Soybean meal	Dry matter (%)	Crude protein (%)	Crude lipid (%)
High-value soybean meal	69.6 ± 1.16	89.2 ± 0.35*	86.6 ± 0.42
Commercial soybean meal	69.6 ± 0.75	86.7 ± 0.21†	85.6 ± 0.25
t-test†			
F value	0.001	37.955	4.556
P value	0.976	0.004	0.100

*Values are presented as means ± SEM. Values in the same column with different superscripts are significantly different from each other ($P < 0.05$).
†*t*-test, Independent-samples *t*-test.

Table 8 Apparent digestibility coefficients (%) of dry matter, crude protein and lipid of fish fed diets with graded levels of soybean proteins from CSM or HVS meal*

Diets no. (substitution level)	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Phosphorus (%)
Diet 1 (0)	71.4^{ab}	91.2^{a}	89.5^{a}	70.8^{abc}
Diet 2 (15%)	67.7^{abc}	88.6^{ab}	87.8^{ab}	70.6^{abc}
Diet 3 (30%)	65.0^{abcd}	88.7^{ab}	85.8^{abc}	69.1^{bcd}
Diet 4 (45%)	63.5^{bcd}	86.7^{bc}	83.8^{bc}	65.9^{cd}
Diet 5 (60%)	59.6^{cd}	83.7^{d}	82.5^{c}	64.4^{d}
Diet 6 (15%)	72.2^{a}	88.7^{ab}	89.0^{a}	75.1^{a}
Diet 7 (30%)	66.4^{abcd}	88.8^{ab}	88.1^{ab}	72.1^{ab}
Diet 8 (45%)	63.5^{bcd}	88.5^{abc}	87.1^{abc}	72.7^{ab}
Diet 9 (60%)	57.9^{d}	85.9^{cd}	84.4^{bc}	65.7^{cd}
Pooled SEM	1.0	0.5	0.5	0.9
Two-way ANOVA		*F* value		
Substitution level	14.704	32.426	12.306	3.714
Soybean sources	0.811	7.183	10.745	6.283
Level × sources	1.276	2.156	0.424	0.557
		P value		
Substitution level	0.000	0.000	0.000	0.023
Soybean sources	0.382	0.013	0.004	0.022
Level × sources	0.318	0.133	0.738	0.650

*Values are presented as means ± SEM. Values in the same column with the same superscripts are not significantly different as determined using Tukey' test ($P > 0.05$).
CSM, commercial soybean meal; HVS, high-value soybean meal.

why the growth performance in HVS was better than CSM at the same substitution level.

Conclusion

In general, the growth decreased significantly with increasing dietary soybean protein in both soybean proteins. When the substitution level was 45% or more in CSM, and 60% in HVS, the growth was significantly lower compared with the control group. Therefore, 30% protein could be replaced by CSM, and 45% by HVS in the diets of juvenile Japanese seabass. The higher substitution level for HVS compared with CSM was probably due to better nutritional values such as available phosphorous levels.

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