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Comparison of high-protein soybean meal and commercial soybean meal partly replacing fish meal on the activities of digestive enzymes and aminotransferases in juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828)

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Abstract

This study was conducted to compare the effects of fish meal (FM) replacement by two types of soybean meals (SBMs) on activities of digestive enzyme, intestinal brush border enzyme and aminotransferases of juvenile Japanese seabass (initial weight 8.3 ± 0.2 g). Nine isonitrogenous (crude protein 44%) and isoenergetic (20 kJ g^{-1}) experimental diets replacing 0 (the control), 15%, 30%, 45% and 60% FM protein by high-protein SBM (HSB) or commercial SBM (CSB) were formulated. The concentrations of anti-nutritional factors (ANFs) in HSB were relatively lower compared with CSB ($P < 0.05$). After 10-week feeding experiment, activities of digestive enzymes, intestinal brush border enzymes and aminotransferases in liver decreased with increasing dietary SBM. When the substitution level was above 30% with CSB and 60% with HSB, activities of pepsin, disaccharidase in the intestinal brush border membrane and aspartate aminotransferase (AST) in liver were significantly lower than the control. However, activities of alanine aminotransferase (ALT) and AST in serum were significantly higher than the control when the substitution level was above 30% with CSB and 60% with HSB. Regression analysis showed that effects of FM substitution by HSB on digestion and metabolism of juvenile seabass were relatively smaller compared with CSB.

Keywords: soybean meal, juvenile Japanese seabass, digestive enzymes, brush border enzymes, aminotransferases

Introduction

The nutritional value of protein source is primarily influenced by its digestibility and amino acid (AA) profile. In this respect, fish meal (FM) is generally considered as an excellent protein source in aquaculture feeds. However, inclusion of high percentages of FM in fish diets increases costs. Soybean meal (SBM), one of the most promising plant protein sources to replace FM, has been usually used to replace FM because it has relatively well-balanced AA composition and is widely available (Dabrowski, Poczyczynski, Kock & Berger 1989; Storebakken, Refstie & Ruyter 2000; Leenhouwers, Adjei-Boateng, Verreth & Schrama 2006). However, SBM quality varies in terms of anti-nutritional factor (ANF) concentration, protein content, nutrient digestibility etc. High-protein SBM (HSB) is rich in protein and low in ANFs and therefore commonly used in commercial feeds for swine, beef, dairy cattle, poultry, fish and shrimp (Liang, Kotowski & Chi 2005). To date, effects of partial replacement of FM with HSB vs. CSB on the digestion and absorption of the feed in fish have not been further studied.

Feed ingredient would be effective only when it could be well digested and absorbed. Digestion and

absorption of nutrients directly depend on the activities of digestive–absorptive enzymes in the alimentary tract. Hence, measuring the activity of digestive enzymes provides insight into feed utilization and nutrient digestibility (Rungruangsak-Torrissen 2001; Fountoulaki, Alexis, Nengas & Venou 2005). The activity of digestive and absorptive enzymes in the brush border membrane of intestinal epithelia cells was significantly reduced with increasing SBM levels in the diet of Atlantic salmon (*Salmo salar* L.) (Bakke-McKellep, Press, Baeverfjord, Krogdahl & Landsverk 2000; Krogdahl, Bakke-Mckellep & Baeberfjord 2003). In addition, high inclusion levels of SBM in the diet reduced the digestion of macronutrients, absorption of intact protein and amino acids, and reabsorption of endogenous digestive components (e.g. taurine) (Dabrowski & Dabrowska 1981; McLean, Rbnsholdt, Sten & Najamuddin, 1999; Nordrum, Krogdahl, Rbsjb, Olli & Holm 2000).

Japanese seabass is a carnivorous species, which has been widely cultured in China because of its delicious meat and rapid growth. In a previous experiment, the effects on growth of partial replacement of FM with HSB or commercial SBM (CSB) in Japanese seabass have been investigated (Li, Ai, Mai, Xu & Cheng 2011). This study was conducted to compare the effects of fish meal replacement with two types of soybean meal on the activities of digestive enzymes, intestinal brush border enzymes and aminotransferases in juvenile Japanese seabass.

Materials and methods

Diets and fish

HSB, obtained from RENESSEN Corp of United States, contained 61% (dry weight) crude protein. HSB was produced by dehulling, cracking, heating and flaking soybeans, and the oil was reduced by the use of hexane or homologous hydrocarbon solvents. The extracted flakes were cooked and ground into a meal, and toasted to inactivate certain ANFs. CSB, obtained from the commercial market, contained 53% (dry weight) crude protein. The diets and experimental design were given in Li *et al.* (2011). In brief, nine isonitrogenous (crude protein 44%) and isoenergetic (20 kJ g^{-1}) practical diets were formulated to replace 0 (the control), 15%, 30%, 45% and 60% of the FM protein by HSB or CSB (Table 1). The diets were named CSB0 (the control), CSB15, CSB30, CSB45, CSB60, HSB15, HSB30, HSB45 and HSB60 respectively. All diets were produced using a twin-screw extruder [F-26(II), South China University of Technology, China] and were stored at −20°C until used.

Japanese seabass was obtained from Ningbo Fisheries Corporation, Zhejiang, China. Before the start of the experiment, the juvenile seabass was fed the control diet during a 10-day acclimation period. At the start of the experiment, the fish were fasted for 24 h. One thousand and eighty fish averaging 8.3 ± 0.2 g were hand sorted and distributed randomly into 36 seawater floating cages (1.5 × 1.5 × 2.0 m). Each diet was randomly assigned to four replicate cages. 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 22.2 ± 1.9°C, the salinity was 26–30 mg L^{-1} and the dissolved oxygen content was 6.30 ± 0.05 mg L^{-1}.

Analyses and measurement

Chemical analyses of ANFs

The ANFs, including trypsin inhibitor, phytic acid, soya isoflavone and soyasaponins in HSB and CSB, were analysed. Trypsin inhibitor was analysed according to the method of Kakade, Rackis, McGhee and Puski (1974) and Hammerstand, Black and Glover (1981), using N-benzoyl-DL-arginine -*p*-nitroanilide (BAPNA, Fluka Chemicals®) as substrate. Phytic acid was analysed using the modified anion exchange column method of Nahapetian and Bassirt (1975) and Ellis and Morris (1983), and soyasaponins using GLC method (Ebrahimzadeh & Niknam 1998). HPLC analysis was used to determine soya isoflavone content based on the method of Bala and Uniyal (2002).

Sample collection

At the termination of the experiment, the fish were fasted for 24 h and harvested. Five fish per cage were anaesthetized with eugenol (1:10000) (Shanghai Reagent Corporation, China), and blood samples were collected from the caudal vasculature using a 1-mL syringe and 27-gauge needle, and allowed to clot at room temperature for 4 h (Hardic, Fletcher & Secombes 1990). Following centrifugation (836 × *g*, 10 min, 4°C), the serum was removed and frozen at −80°C until assayed.

The liver, stomach and whole intestinal tract contents were obtained from the above five fish,

Table 1 Formulation and proximate composition of experiment diets containing CSB and HSB (% dry matter)

	Diets containing CSB[2]					Diets containing HSB[3]			
Ingredient	**CSB0**	**CSB15**	**CSB30**	**CSB45**	**CSB60**	**HSB15**	**HSB30**	**HSB45**	**HSB60**
Fish meal[1]	52.0	44.2	36.4	28.6	20.8	44.2	36.4	28.6	20.8
soybean meal	0.0	10.5	21.0	31.5	42.0	9.1	18.3	27.4	36.6
Beer yeast[1]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Menhaden fish oil	1.0	2.2	3.5	4.8	6.0	2.2	3.5	4.7	6.0
Soybean oil	1.0	0.9	0.8	0.7	0.6	0.9	0.8	0.7	0.6
Wheat meal[1]	26.0	24.0	23.0	21.0	18.0	23.5	21.0	19.0	17.0
Vitamin premix[4]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Starch[5]	5.00	3.80	1.90	0.50	0.08	4.9	4.8	4.6	4.3
Attractant[6]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lecithin	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Antioxidant[7]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Yttrium oxide	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
AA premix	0.42	0.53	0.65	0.82	1.00	0.64	0.86	1.10	1.37
Cellulose[8]	3.99	2.78	1.46	0.69	0.03	3.57	3.15	2.51	1.84
Proximate composition	91.3	91.2	91.5	91.2	91.0	91.2	92.3	91.0	91.0
Dry matter (%)									
Crude protein (%)	44.4	44. 5	44.2	44.0	43.8	44.0	44.3	44.1	44.6
Crude lipid (%)	11.2	11.8	11.6	11.5	11.8	11.1	11.2	11.7	11.9
Digestible phosphorus (%)	0.93	0.82	0.72	0.61	0.57	0.90	0.83	0.71	0.64

[1]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; 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

[2]CSB, obtained from commercial market, crude protein, 52.7% dry matter, crude lipid, 1.3% dry matter

[3]HSB, obtained from Renessen in Illinois state of United States, 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;

[4]Commercial mixture supplied by Qingdao Marine Science & Technology Bio-Tech (Shandong, China), same as described by Li *et al.* (2011)

[5]Starch: Corn starch

[6]Attractant: Dimethyl-β-propiothetin-DMPT

[7]Antioxidant: Ethoxyquin, obtained from Qingdao Master Biotechnology

[8]Cellulose: Microcrystalline cellulose

and chyme was removed from the gut and stomach using distilled water. Each section of the organ in five fish per cage was pooled and frozen in liquid nitrogen, stored at −80°C to analyse the activities of digestive enzymes and aminotransferases.

Five additional fish per cage were anaesthetized for collection of intestinal samples. The whole intestine was cut open and rinsed in saline water. The mucosa was scraped off the intestine wall with a plastic spatula, and the five samples per cage were pooled frozen in liquid nitrogen and stored at −80°C to analyse the activities of intestinal brush border enzymes (Krogdahl *et al.* 2003).

Digestive enzyme activities

The liver, stomach and intestine samples were accurately weighed, then homogenized in ice-cold distilled water in the proportion of 1:5 (w/v). Following centrifugation (1800 × *g*, 30 min, 4°C), the supernatants were removed and kept at 4°C for analysis.

The pepsin activity was measured using casein hydrolysis (modified by Walter 1984). One unit of protease activity was defined as 1 μg tyrosine liberated by hydrolyzing casein in 1 min at 37°C. The lipase activity was measured as described by Winkler and Stuckman (1979). The principle of the assay is the colorimetric estimation of *p*-nitrophenol (*p*NP) released due to enzymatic hydrolysis of *p*-nitrophenyl palmitate (*p*NPP) at 410 nm. One enzyme unit is defined as 1 nmol of *p*-nitrophenol enzymatically released from the substrate in 1 min at 37°C. The amylase activity was determined using the starch-hydrolysis method, described by Robyt and Whelan (1968). One unit

of amylase activity (U mg protein^{-1}) was defined to hydrolyze 0.1 mg of starch in 30 min at 37°C. Specific activity was expressed as enzyme activity per mg protein. The protein concentration of the supernatant solutions was determined using the method of Lowry, Rosebrough, Farr and Randall (1951), using bovine serum albumin as the standard.

Enzyme activities in the intestinal brush border membrane

The intestinal mucosa samples were accurately weighted, and homogenized in ice-cold distilled water in the proportion of 1:3 (w/v), following centrifugation (4100 × *g*, 10 min). The supernatants were removed and kept at 4°C to analyse.

The alkaline phosphatase (ALP) and leucine aminopeptidase (LAP) activities were determined colorimetrically as previously described by Krogdahl *et al.* (2003). The activities of disaccharidase (maltase, lactase and sucrase) were analysed according to the method described by Siddons (1972) and Thomsen and Tasman (1982), using maltose, lactose and sucrose, respectively, as substrates. Enzyme activities were expressed as μmol substrate hydrolyzed per mg protein in the homogenate.

Activities of aminotransferases in liver and serum

Crude extracts of liver for assaying activities of aminotransferases were obtained using homogenization of frozen tissue in ice-cold 0.7% saltwater. Following centrifugation (10,000 × *g*, 20 min, 4°C), activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in liver supernatants and serum were measured using specific analytical procedures and commercially available kits (alanine aminotransferase reagent kit for ALT analysis and aspartate aminotransferase reagent kit for AST analysis, produced by Jiancheng engineering Institute, Nanjing, China).

Statistical analyses

Data were analysed using one-way and two-way analysis of variance to determine if there were significant differences ($P < 0.05$) due to the dietary levels of substitution, type of SBM or the interaction. *T*-test (variance ratio) was used to determine the significant difference in ANFs between two types of soybean meals. The level of significance was chosen at $P < 0.05$ and the results were presented as the mean ± S.E.M. (standard error of means). Regression analyses were used to investigate the effects of increasing levels of dietary HSB or CSB on the activities of LAP, ALP, AST and ALT.

Results

ANFs of two SBMs

Trypsin inhibitor was 11.14 mg g^{-1} in HSB and 10.35 mg g^{-1} in CSB, with no significant difference between two meals. The concentration of phytic acid in CSB (0.62%) was significantly higher compared with HSB (0.57%). Both soya isoflavone (1.15 mg kg^{-1}) and soyasaponins (0.34%) in HSB were significantly lower than those in CSB (soya isoflavone, 1.55 mg kg^{-1}; soyasaponins, 0.41%) ($P < 0.05$). Soya isoflavone are mainly composed of isoflavoues aglycone, genistein and glycetein, and the concentrations in CSB were all significantly higher than in HSB (Table 2).

Table 2 The ANFs of normal CSB and HSB[1]

Soybean meal	HSB[2]	CSB[3]	*T*-test[4] t value	*T*-test[4] *P* value
Trypsin inhibitors (mg g^{-1})	11.14 ± 0.30	10.35 ± 0.24	−2.065	0.175
Phytic acid (%)	0.57 ± 0.01^{b}	0.62 ± 0.00^{a}	4.294	0.049
Soyasaponins (%)	0.34 ± 0.01^{b}	0.41 ± 0.00^{a}	7.501	0.000
Isoflavone (mg g^{-1})	1.15 ± 0.03^{b}	1.55 ± 0.09^{a}	4.971	0.003
Isoflavoues aglycone[5] (mg g^{-1})	0.48 ± 0.01^{b}	0.55 ± 0.02^{a}	2.796	0.031
Glycetein[5] (mg g^{-1})	0.06 ± 0.00^{b}	0.12 ± 0.01^{a}	6.447	0.001
Genistein[5] (mg g^{-1})	0.17 ± 0.01^{b}	0.29 ± 0.02^{a}	4.745	0.003

[1]ANFs, anti-nutritional factors; Values are presented as means with pooled S.E.M., Values in the same column with different superscripts are significantly different from each other ($P < 0.05$).
[2]HSB: high-value soybean meal.
[3]CSB: commercial soybean meal.
[4]*T*-Test: Independent Samples *T*-Test.
[5]Isoflavoues aglycone, genistein and glycetein are three major compositions of soy isoflavone.

The activities of digestive enzymes in the alimentary tract

The activities of digestive enzymes in the alimentary tract of fish decreased with increasing dietary SBM (Table 3). The activities of proteases in liver were significantly lower than the control when the substitution level was 60% in both HSB and CSB. When the substitution level was 45% or higher with CSB, the activities of pepsins were significantly lower compared with the control. However, there were no significant differences in the activity of pepsin among fish fed diets with less than 60% HSB. The activities of lipase in the intestine of fish fed the diet with 15% protein from HSB were higher than the control. There were no significant differences in activity of protease of the intestine, activity of lipase of the liver and activity of amylase of the stomach and intestine among dietary groups. There was no interaction between SBM type and inclusion levels in activities of digestive enzymes in the alimentary tract of juvenile Japanese seabass.

The activities of enzymes in the intestinal brush border membrane

Regression analysis demonstrated a linear decrease in LAP activity as the inclusion level of SBM increased ($R^2 = 0.967$, $P = 0.003$ for HSB; $R^2 = 0.947$, $P = 0.005$ for CSB) (Fig. 1). The activities of ALP decreased with increasing dietary SBM. When the substitution level was 60% with CSB, the ALP activity was significantly lower compared with fish fed the control diet. However, no significant differences were observed in ALP activity among fish fed diets with graded levels of HSB (Table 4). The regression equation between the activities of ALP and substitution levels of dietary SBMs was $y = -0.018x + 2.271$ ($R^2 = 0.836$, $P = 0.029$) for HSB and $y = -0.027x + 2.362$ ($R^2 = 0.939$, $P = 0.006$) for CSB. This showed that ALP activity in fish fed diets with FM replacement of HSB decreased more slowly compared with CSB diets (Fig. 2). The enzyme activities of disaccharidase (maltase, lactase and sucrase) of the intestinal brush border membrane in fish were

Table 3 The activity of digestive enzymes in alimentary tract of juvenile Japanese seabass fed diets with graded levels of soybean proteins from CSB[1] or HSB[2,3]

Diets no. (substitution level)	Protease (U mg^{-1} protein)			Lipase (U mg^{-1} protein)		Amylase (U mg^{-1} protein)		
	liver	stomach	intestine	liver	intestine	liver	stomach	intestine
CSB0	0.19^{a}	43.22^{a}	1.57	5.25	47.23^{bc}	2.33^{ab}	2.24	2.64
CSB15	0.17^{ab}	31.95^{ab}	1.60	4.63	55.40^{b}	1.98^{ab}	1.79	3.04
CSB30	0.15^{abc}	31.26^{ab}	1.56	4.68	46.07^{bc}	1.36^{ab}	1.01	2.12
CSB45	0.12^{abc}	28.67^{b}	0.54	5.01	39.87^{cd}	1.01^{b}	1.00	2.21
CSB60	0.09^{c}	28.07^{b}	0.33	4.93	29.29^{d}	0.91^{b}	0.72	1.97
HSB15	0.17^{ab}	35.81^{ab}	1.82	5.31	77.27^{a}	3.49^{a}	1.42	2.67
HSB30	0.15^{abc}	33.31^{ab}	0.85	4.52	50.19^{bc}	1.53^{ab}	1.66	2.39
HSB45	0.14^{abc}	29.43^{ab}	0.67	4.50	49.80^{bc}	1.38^{ab}	1.13	2.53
HSB60	0.11^{bc}	28.55^{b}	0.61	4.20	44.71^{bc}	1.25^{ab}	0.99	2.19
Pooled S.E.M.	0.01	1.19	0.16	0.10	0.01	0.18	0.15	0.16
Two-way ANOVA	*F* value							
Substitutio n level	6.837	5.574	2.097	0.862	22.601	4.546	1.696	0.901
Soybean meals	1.066	0.897	0.107	1.758	16.356	2.375	0.122	0.579
Level × meals	0.776	0.145	0.771	2.857	3.216	0.997	0.626	0.364
	P value							
Substitution level	0.001	0.004	0.123	0.504	0.000	0.009	0.190	0.482
Soybean	0.315	0.356	0.747	0.201	0.001	0.139	0.730	0.456
Level × meals	0.522	0.866	0.477	0.064	0.064	0.415	0.545	0.779

[1]HSB: high-value soybean meal.
[2]CSB: commercial soybean meal.
[3]values are presented as means ± S.E.M. Values in the same column with the same superscripts are not significantly different determined using Turkey' test ($P > 0.05$).

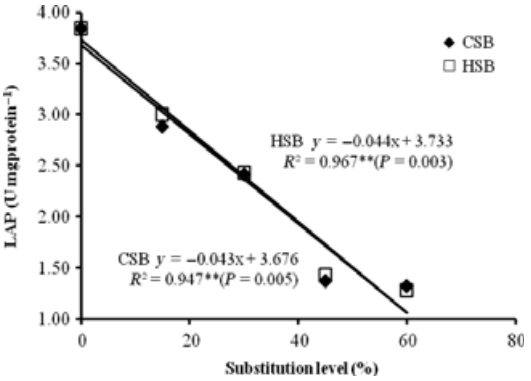

Figure 1 Regression between the two soybean proteins substitution levels (x) and activities of leucine aminopeptidase (LAP) (y) in intestinal brush border membrane of juvenile Japanese seabass. Treatments marked (**) are significantly different from the control group at $^{**}P < 0.01$.

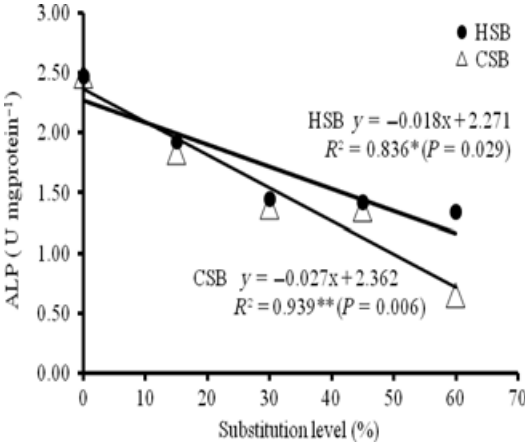

Figure 2 Regression between the two soybean proteins substitution levels (x) and activities of alkaline phosphatase (ALP) (y) in intestinal brush border membrane of juvenile Japanese seabass. Treatments marked (* or **) are significantly different from the control group at $^{*}P < 0.05$ or $^{**}P < 0.01$.

Table 4 The effects of the two soybean meals, HSB[1] and CSB[2], on the activities of selected enzymes in intestinal brush border membrane of juvenile Japanese seabass[3].

Diets no. (substitution level)	ALP[4] (U mgprotein^{-1})	LAP[5] (U mgprotein^{-1})	Maltase (U mgprotein^{-1})	Sucrase (U mgprotein^{-1})	Lactase (U mgprotein^{-1})
CSB0	2.47^{a}	3.84	8.75^{a}	9.13^{a}	0.59^{a}
CSB15	1.84^{ab}	2.89	6.87^{ab}	8.05^{ab}	0.52^{ab}
CSB30	1.38^{ab}	2.42	6.73^{ab}	6.68^{abc}	0.43^{abc}
CSB45	1.36^{ab}	1.37	6.15^{b}	4.49^{c}	0.33^{bc}
CSB60	0.65^{b}	1.28	5.02^{b}	4.46^{c}	0.21^{c}
HSB15	1.92^{a}	3.00	7.06^{ab}	9.40^{a}	0.67^{a}
HSB30	1.45^{ab}	2.43	6.91^{ab}	7.59^{ab}	0.46^{ab}
HSB45	1.42^{ab}	1.43	6.85^{ab}	6.64^{abc}	0.45^{abc}
HSB60	1.34^{ab}	1.33	5.77^{b}	5.68^{bc}	0.32^{bc}
Pooled S.E.M.	0.11	0.31	0.20	0.36	0.03
Two-way ANOVA	*F* value				
Substitution level	7.371	1.717	8.769	20.335	19.478
Soybean meals	1.730	0.003	2.117	12.941	11.361
Level × meals	0.913	0.002	0.267	0.424	0.650
	P value				
Substitution level	0.001	0.182	0.000	0.000	0.000
Soybean meals	0.204	0.960	0.159	0.002	0.008
Level × meals	0.453	1.000	0.849	0.739	0.603

[1]HSB: high-value soybean meal.
[2]CSB: commercial soybean meal.
[3]Values are presented as means with pooled S.E.M., Values in the same column with the same superscripts are not significantly different determined using Tukey' test ($P > 0.05$).
[4]ALP: Alkaline phosphatase
[5]LAP: Leucine aminopeptidase

inversely correlated with dietary HSB and CSB. When the substitution level was 45% or higher with CSB, and 60% with HSB, activities of maltase, lactase and sucrase were significantly lower compared with the control diet. There was no interaction between soybean meal types and substitution level on the activities of enzymes in the intestinal brush border membrane.

The activities of aminotransferases in the liver and serum

The activities of ALT and AST in liver decreased with increasing dietary SBM (Table 5). When the substitution level was above 30% with CSB, and 60% with HSB, the activities of AST in liver were significantly lower than the control ($P < 0.05$). However, no significant differences were observed in the activities of ALT in liver among fish fed each diet ($P > 0.05$). Regression analysis indicated that the activities of AST or ALT in fish fed CSB diets decreased more quickly compared with HSB diets because of a lower slope (Fig. 3). The change of ALT and AST in serum was different from those in liver. Both activities of ALT and AST in serum increased with increasing dietary SBM. When the substitution level was above 30% with CSB, and 60% with HSB, the activities of ALT and AST in serum were significantly higher than the control ($P < 0.05$). There was an interaction between soybean types and substitution level on activities of AST in serum.

Table 5 ALT, AST in liver and serum of fish fed diets with graded levels of soybean proteins from CSB[1] or HSB[2, 3]

Diets no. (substitution level)	Liver (U g protein^{-1})		Serum(U L^{-1})	
	AST[4]	ALT[5]	AST	ALT
CSB0	344.9^{a}	695.4	1531.7^{c}	202.6^{b}
CSB15	296.1^{ab}	555.0	1688.2^{bc}	422.7^{b}
CSB30	277.9^{abc}	446.8	2441.9^{abc}	476.8^{b}
CSB45	228.7^{bc}	434.3	3540.0^{a}	647.4^{a}
CSB60	197.8^{c}	389.8	3823.3^{a}	1117.1^{a}
HSB15	310.1^{ab}	560.6	2667.8^{abc}	348.3^{b}
HSB30	307.1^{ab}	491.1	2692.8^{abc}	387.7^{b}
HSB45	269.1^{abc}	469.9	2802.5^{abc}	644.2^{ab}
HSB60	255.2^{bc}	465.6	3095.6^{ab}	663.6^{a}
Pooled S.E.M.	9.186	26.187	178.806	59.609
Two-way ANOVA	*F* value			
Substitution level	9.192	4.034	11.573	15.217
Soybean meals	7.251	0.602	0.087	5.599
Level × meals	0.507	0.089	4.422	2.223
	P value			
Substitution level	0.000	0.015	0.000	0.000
Soybean meals	0.013	0.447	0.774	0.036
Level × meals	0.682	0.965	0.026	0.138

[1]HSB: high-value soybean meal.
[2]CSB: commercial soybean meal.
[3]Values are presented as means with pooled S.E.M., Values in the same column with the same superscripts are not significantly different determined using Tukey' test ($P > 0.05$).
[4]AST: aspartate aminotransferase
[5]ALT: alanine aminotransferase

Discussion

The concentration of saponin in HSB and CSB used in this study was 0.34% and 0.41%, respectively, which was lower than that (0.43–0.67%) reported by Ireland, Dziedzic and Kearsley (1986). Tsai and Huang (1999) found that the natural level of isoflavones in soy protein concentrate (SPC) or soy protein isolate (SPI) was approximately 270–1850 mg kg^{-1}. The concentration of isoflavones in HSB (1150 mg kg^{-1}) and CSB (1550 mg kg^{-1}) was in range of the above interval. Phytic acid in SBM was usually 0.6% (Peisker 2001; Hanssen 2003), which was similar to the results (0.57–0.62%) of this experiment.

The digestibility and utilization of nutrient mainly depends on the activities of digestive enzymes in the gastrointestinal tract. In this study, the activities of digestive enzymes in the alimentary

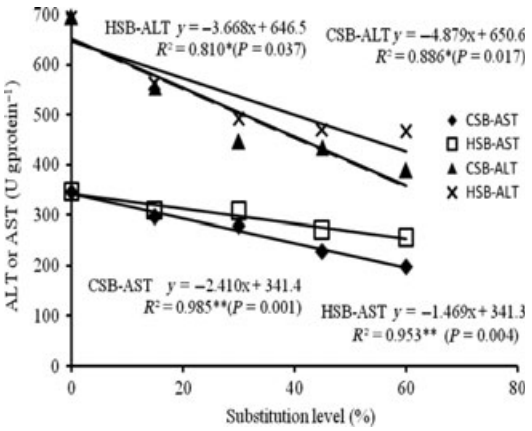

Figure 3 Regression between the two soybean proteins substitution levels (x) and activities of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) (y) in liver of juvenile Japanese seabass. Treatments marked (* or **) are significantly different from the control group at *$P < 0.05$ or **$P < 0.01$.

tract decreased with increasing dietary SBM, which agreed well with the previously published results regarding digestibility of nutrients (Li *et al.* 2011). These results indicated that higher levels of dietary SBM decreased the digestibility of juvenile Japanese seabass. The presence of ANFs in SBM could reduce the activity of digestive enzymes, thereby reducing the digestibility of the feedstuff (Leenhouwers *et al.* 2006; Deng, Mai, Ai, Zhang, Tan, Xu & Liufu 2010). Trypsin inhibitor and phytic acid present in soy products has been reported to have a negative effect on protease activity (Denstadli, Skrede, Krogdahl, Sahlstrom & StorebakkenT. 2006). Alcohol-soluble components (e.g. saponins, isoflavones) can also reduce nutrient digestibility (Refstie, Svihus, Shearer & Storebakken 1999; Romarheim, Skrede, Gao, Krogdahl, Denstadli, Lilleeng & Storebakken 2006). Fish fed diets containing HSB at the same FM substitution level showed higher activities of digestive enzymes compared with CSB diets. This could attribute to relatively lower ANFs in HSB, such as phytic acid, saponins and isoflavones compared with CSM. The previously published paper showed that the nutritional value of HSB was better than CSB (Li *et al.* 2011), because the digestibility of juvenile Japanese seabass fed HSB diet with the same FM substitution level was improved compared with CSB.

Disaccharidases are a glycoprotein of the intestine brush border membrane that plays an important role in final degradation of carbohydrates before absorption (Harpaz & Uni 1999). In this study, activities of maltase, lactase and sucrose significantly decreased with increasing dietary SBM, indicating that the ability to breakdown carbohydrates of juvenile Japanese seabass decreased when SBM was used as the main protein source. ALP and LAP are considered indicative of enterocyte activity and a marker for the intensity of nutrient absorption of fish (Segner, Oesch, Schmidt & Poeppinghausen 1989; Harpaz & Uni 1999). In this study, activities of ALP and LAP decreased with increasing dietary SBM. Dabrowski *et al.* (1989) and Krogdahl *et al.* (2003) also found that activities of LAP and ALP in the intestinal mucosa of fish fed SBM diet were likely lower than fish fed FM diets due to the lower content of phoshoproteins generally found in vegetable meals compared with FM (Silva, Nicoli, Zambonino-Infante, Gall, Kaushik & Gatesoupe 2010). However, no studies have so far analysed the change trend of activities of LAP or ALP with increasing dietary SBM. In this study, regression analysis showed that the relationships between activities of LAP or ALP and dietary HSB or CSB inclusion were linear, and activities of LAP and ALP in fish fed HSB diets decreased more slowly compared with CSB diets. This can provide evidence that HSB was better protein to replace FM for juvenile Japanese seabass compared with CSB. ANFs (e.g. soya saponins) of SBM could cause pathological changes in the intestine, which lead to a significant reduction in the activities of digestive–absorptive enzymes located on the brush border membrane of enterocytes (Bureau, Harris & Cho 1998; Krogdahl *et al.* 2003). Results of this study found that the activities of digestive–absorptive enzymes for juvenile Japanese seabass fed the diet with HSB at the same substitution level were higher than CSB. This could attribute to the concentrations of ANFs in HSB were relatively lower than in CSB to some extent.

The enzymes of ALT and AST are involved in transamination. The regression analysis demonstrated that activities of ALT and AST in liver of juvenile Japanese seabass were reduced with increasing dietary SBM, which coincides with results for tilapia *Oreochromis niloticus* × *O. aureus* (Lin & Luo 2011) and Atlantic cod *Gadus morhua* L. (Hansen, Rosenlund, Karlsen, Koppe & Hemre 2007). This indicates that protein metabolization was insufficient to increase amino acid level, which in turn influenced this enzyme activity (Trenzado, Morales & Higuera 2006). Aminotransferases in serum can give information on the damage of organs and in particular of liver cells (Kumar, Makkar, Amselgruber & Becker 2010). When liver cells are damaged, aminotransferases including ALT and AST leak into the blood (Racicot, Gaudet & Leray 1975). Significantly higher ALT and AST levels in serum were found in juvenile Japanese seabass fed diets with 45% or more CSB and 60% HSB. These results indicate that fish liver could be damaged to some extent, which could have affected protein metabolism. Aminotransferases can catabolize AA and transfer amino groups to alpha-keto acids (reversible catalysis). When the available AA is deficient, the keto acids may be reduced, thereby reducing the activity of ALT and AST in liver. In this study, AA have been supplemented to meet the IAA requirements based on whole-body AA composition of Japanese seabass, but utilization of crystal amino acids was different from intact protein,

which has been confirmed by the findings of growth performance in this fish (Li *et al.* 2011).

Conclusion

Activities of digestive and metabolism enzymes decreased with increasing dietary SBM. Effects of FM substitution by HSB on digestion and metabolism of juvenile seabass were relatively smaller compared with CSB

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