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Soybean and cottonseed meals are good candidates for fishmeal replacement in the diet of juvenile *Macrobrachium nipponense*

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Abstract *Macrobrachium nipponense* is a new aquaculture species with high market demand and is preferred as food by Asian consumers. However, its nutrient requirements and optimal dietary sources have not been fully evaluated. In the present study, we examined four common plant protein sources (soybean meal, cottonseed meal, rapeseed meal, and peanut meal) as partial replacement for 25% fishmeal protein in five isonitrogenous and isocaloric diets in *M. nipponense* for 8 weeks. The nutritional effects of the four plant protein sources were compared by means of growth performance, feed efficiency, histology, biochemical composition, oxidative stress, total hemocyte count, and in vitro digestion. Prawns fed the diets containing soybean (730.96 ± 33.50%) and cottonseed meals (672.32 ± 74.52%) had higher weight gain than those fed on rapeseed (503.71 ± 50.85%) and peanut (507.79 ± 52.10%) meal diets ($p < 0.05$). Similarly, the feed conversion ratios of prawns fed on soybean (1.66 ± 0.12%) and cottonseed (1.88 ± 0.06%) meal diets were significantly lower than those fed on rapeseed (2.37 ± 0.10%) and peanut (2.77 ± 0.16%) ($p < 0.05$) meal diets. No significant differences were found among groups in the hepatopancreas and intestinal histological characteristics, activities of antioxidant enzymes, and hemocyte number. The amino acid composition in diets and prawn muscles was comparable among groups. Soybean meal showed the highest in vitro

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digestibility. Taken together, soybean and cottonseed meals could be good candidates for partial fishmeal replacement in *M. nipponense* diets.

Keywords *Macrobrachium nipponense* · Fishmeal replacement · Plant protein · Growth · Digestibility · Amino acids component

Abbreviations

SBM	Soybean meal
CSM	Cottonseed meal
RPM	Rapeseed meal
PNM	Peanut meal
WG	Weight gain
FCR	Feed conversion ratio
HSI	Hepatosomatic
SOD	Superoxide dismutase
GSH-Px	Glutathione peroxidase
MDA	Malondialdehyde

Introduction

Macrobrachium nipponense (Oriental river prawn, de Haan 1849) is a new aquaculture species with high market demand (Kutty et al. 2007) and is preferred as food by Asian consumers (Kutty et al. 2009). *Macrobrachium nipponense* is widely distributed in freshwater and low-salinity estuarine regions of China, Japan, and other South-East Asian countries (Yang et al. 2004). Compared with other crustaceans, the nutrient requirements and the optimal dietary sources of *M. nipponense* have not been fully investigated (Yu and Shen 1990; Xu, 2001; Wang et al., 2009a, b; Kong et al. 2014), therefore no specific dietary guidelines for *M. nipponense*. Previous studies have indicated that, the dietary protein requirement of juvenile *M. nipponense* ranges from 36.2 to 42.7% (Yu and Shen 1990; Xie et al. 2007; Zhang et al. 2008). Recently, Zhang et al. (2016) reported that 33% protein and 14% lipid, in a semi-purified diet, could obtain optimum growth performance of *M. nipponense* indicating that relatively high protein level is required for *M. nipponense* diet.

Fishmeal has been widely used as the most important protein source, because of its balanced amino acid (AA) composition, high digestibility, and palatability in aquatic animals' feed formulations. However, fishmeal is becoming increasingly limited due to reduction in global fisheries capture, which has led to a subsequent decline in the aquatic feed industry (Cao et al. 2015). Continued growth and intensification of aquaculture production depends upon the development of sustainable protein sources to replace fishmeal in aquafeeds. Therefore, research on alternative plant proteins to replace fishmeal has gained increasing interest in aquaculture nutrition.

Although plant protein sources have some negative qualities, such as poor palatability, low digestibility, poor utilization of protein and amino acids, presence of anti-nutritional factors, and other potential unknown factors (Gatlin et al. 2007). Fishmeal partial replacement by plant protein sources has been demonstrated not to affect growth and normal physiological characteristics significantly in some aquatic animals (Gómez-Requeni et al. 2004; Alvarez et al. 2007; Minh et al. 2007). A number of plant by-products, such as soybean meal (SBM),

cottonseed meal (CSM), rapeseed meal (RPM), and peanut meal (PNM), have been studied in the diets of some crustaceans as potential candidates for fishmeal replacement (Lim 1997; Alvarez et al. 2007; Luo et al. 2011; Jiang et al. 2012). Dietary replacement of fishmeal by plant protein sources, like SBM, CSM, and RPM, has been increasing in the aquaculture industry because of their low price, high market availabilities, and sufficient protein contents in aquatic species diets (Hardy 1995).

However, in *M. nipponense*, only Ding et al. (2015a, b) reported that 25% replacement of fishmeal by fermented soybean meal would not affect the growth and immunological characteristics, while fishmeal replacing effects of other plant protein sources like CSM, RPM, and PNM have not been thoroughly investigated. Despite the fact that, the optimal dietary protein requirement of *M. nipponense* is relatively high, it can adapt to a plant-based diet because of its omnivorous feeding habit. Thus, fishmeal partial replacement by plant protein source in *M. nipponense* is important for practical feed formulation to ensure its sustainability.

The present study was designed to replace 25% of fishmeal protein by using four plant protein sources (SBM, CSM, RPM, and PNM). The study evaluated the nutritional effects of these plant protein sources in *M. nipponense* by assessing growth performance, feed efficiency, body composition, digestibility, oxidative stress, hemocyte count, and in vitro digestibility in order to select optimal plant protein sources for the fishmeal replacement.

Materials and methods

Experimental diets

Five isonitrogenous and isocaloric diets were formulated (Table 1). The control diet contained fishmeal as the unique protein source. In the other four experimental diets, 25% of fishmeal was replaced by either SBM, CSM, RPM, or PNM. The total dietary protein contents in the five diets were set at approximately 35%. Corn starch was used as the carbohydrate source for all the five diets. The lipid source was the mixture of fish oil and soybean oil (2:1 *w*/*w*), which promotes growth and prevents precocity in *M. nipponense* (Ding et al. 2014). All the ingredients were thoroughly mixed with oil mixtures, and water was added to produce a stiff dough. The dough was then processed into 3-mm diameter pellets, air dried at room temperature until the moisture content was less than 10%. After drying, the dough was broken up and sieved into proper pellet size depending on the prawn size and stored at − 20 °C until required for feeding experiment.

Experimental procedures

Juvenile *M. nipponense* were obtained from a commercial farm (Bangda Ecological Agriculture Company, Hu Zhou, China). The prawns were cultured into ten 300-L square plastic tanks filled with water to 250 L, each containing 200 prawns for 2 weeks to acclimate them to the experimental conditions. The water for rearing the prawns was obtained from a nearby river. The water in the tanks was obtained by pumping from the nearby river. The water was maintained in a stock tank and aerated for at least 3 days before it was allowed to enter into the culture system to ensure optimum water quality parameters for the growth and survival of the prawns. Water temperature, dissolved oxygen,

Table 1 The formulation and proximate analysis of the experimental diets (%)

Ingredients (%)	Test diets				
	FM	SBM	CSM	RPM	PNM
Fishmeal	57	43	43	43	43
Soybean meal[#]	0	19.5	0	0	0
Cottonseed meal[#]	0	0	16.5	0	0
Rapeseed meal[#]	0	0	0	22	0
Peanut meal[#]	0	0	0	0	19.5
Corn starch	21	21	21	21	21
Fish oil: soybean oil(2/1)	4.3	5.3	5.4	5.2	5.3
Soybean lecithin	0.5	0.5	0.5	0.5	0.5
Cholesterol	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.5	0.5	0.5	0.5	0.5
Mineral premixs[1]	3	3	3	3	3
Vitamin premixs[2]	2	2	2	2	2
Cellulose	8.2	1.7	4.6	0	1.7
Carboxymethylcellulose sodium	3	3	3	3	3
Proximate composition (% in dry matter)					
Crude protein	36.47	35.18	35.40	34.84	36.71
Crude lipid	14.39	14.92	13.04	13.48	14.05
Gross energy (KJ/g)	15.46	15.37	15.29	15.35	15.28

[#] The percentage of fishmeal replacement was set as 25% fishmeal protein by plant source protein

[1] Mineral premix (mg/g premix): KCl 28; $MgSO_4.7H_2O$ 100; NaH_2PO_4 215; KH_2PO_4 100; $Ca(H_2PO_4)_2.H_2O$ 265; $CaCO_3$ 105; $C_6H_{10}CaO_6.5H_2O$ 165; $FeC_6H_5O_7.5H_2O$ 12; $ZnSO_4.7H_2O$ 4.76; $MnSO_4.H_2O$ 1.07; $AlCl_3.6H_2O$, $CuCl_2$. $2H_2O$, 0.24; $CoCl_2.6H_2O$ 1.4; KI, 0.23; a-cellulose, 2.15

[2] Vitamin premix (mg/g premix): vitamin A, 1.0; vitamin D3, 0.63; vitamin E, 10; vitamin K3, 1.8; niacin, 5; riboflavin, 2.63; pyridoxine,1; aminobenzoic acid, 5; thiamine, 0.75; D-calcium pantothenate, 5; biotin, 0.75; folic acid, 0.19; vitamin B12, 0.15; inositol, 60.; a-cellulose, 905.75

and ammonia nitrogen were 26.5 to 29 °C, >6.5 mg L^{-1}, and <0.1 mg L^{-1}, respectively. During acclimation, prawns were hand-fed twice a day to apparent visual satiation by using a commercial diet containing 38% protein. After acclimation period, visually healthy prawns (no signs and symptoms of disease) of comparable similar sizes (0.16 ± 0.03 g) were randomly distributed into twenty 300L static water tanks filled with 250 L water, each containing 45 prawns per tank (four replicates for each dietary group). Water quality parameters were maintained at similar conditions to those used during the acclimation period. About 30% of water in each tank was renewed daily by using mechanically filtered river water. During the whole trial, the water in each tank was continuously aerated using air tubes. Prawns were exposed to a natural photoperiod of 10/14 h dark/light cycle and hand-fed to apparent visual satiation twice daily (08:30 and 16:30) for 56 days. Wasted feed and feces were collected from tanks with a rubber pipe every morning and were dried for feed intake calculation. During the experiment, we check the prawn mortality on daily basis and dead prawns were removed as observed.

Determination of growth performance, survival rate, and feed efficiency

At the end of the experiment, the prawns were starved for 24 h before sampling. All survived prawns were counted and group weighed for calculation of weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio, and survival rate using the following formulae as adopted

from Wang et al. (2006), Ding et al. (2017), Gu et al.(2017), and Méndez-Martínez et al. (2017).

$$\text{Survival rate (SR)} = 100 \text{ x } \left[\frac{\text{Final number of shrimp}}{\text{Initial number of shrimp}}\right]$$

$$\text{Weight gain (WG)} = 100 \text{ x } \left[\frac{\text{Final mean weight-Inital mean weight}}{\text{Initial mean weight}}\right]$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry feed intake}}{\text{Mean wet weight gain}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Mean wet weight gain}}{\text{Protein intake}}$$

Biochemical analysis

The experimental diets and prawn samples were analyzed in triplicate for proximate composition following standard methods (AOAC 1995). Moisture was determined by drying in an oven at 105 °C to a constant weight. Samples used for ash determination were incinerated overnight in a muffle furnace at 550 °C.

Muscle was lyophilized and hydrolyzed for the measurement of total amino acid composition. The amino acid composition of the diets and muscles was determined after acid hydrolysis using the L-8900 amino acid analyzer (Hitachi, Japan). Crude lipids in the whole body were measured by using the ether extraction method using the Soxtec system (2055 Soxtec Avanti; Foss Tecator, Hoganas, Sweden).

Oxidative stress analysis and hematoxylin and eosin staining

Whole prawn, muscle, and hepatopancreas were collected, weighed, and stored at − 80 °C. Hepatopancreas was used for measurement of antioxidant enzymes. The activities of antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) content in hepatopancreas, were measured using commercial kits (Jiancheng Biotech Co., Nanjing, China) according to manufacturer's instructions. The hepatopancreas weight was used to estimate hepatosomatic index using the formula:

$$\text{Hepatosomatic index (HSI)} = 100 \text{ x } \left[\frac{\text{Hepatopancreas weight}}{\text{Fish body weight}}\right]$$

The intestinal tracts and hepatopancreas were fixed in 4% polyformaldehyde for histological observation. Hepatopancreas and intestines were isolated for histological study using hematoxylin and eosin (H and E) staining (Wang et al. 2014).

Total hemocyte count

Hemolymph was collected as described in Yang et al. (2004). Total hemocyte counts (THC, cells ml^{-1}) were performed using a Burker hemocytometer. The hemolymph blood count was

immediately done under the binocular microscope. Counts were performed in triplicate and mean and standard error were calculated.

Estimation of digestibility

Besides the feeding trial, in vitro digestibility of the five protein sources was individually measured as described previously (Wang et al., 2009a, b). The hepatopancreas and intestine were dissected from *M. nipponense*. Thawed samples were pooled, diluted 1:10 (*w*/*v*, distilled water), and homogenized on ice. The homogenate was centrifuged at 4 °C at a speed of 10,000×g for 20 min and the upper lipid layer was discarded. The supernatant was used as enzyme solution for measurement of in vitro digestibility. The in vitro hydrolysis of protein sources was measured by weighing 0.5 g of different protein sources and was added into a 100-ml vessel containing 47.5 ml NaH_2PO_4-Citrate buffer (pH 7.5) at 37 °C. The reaction was started by adding 2.5 ml of enzyme solution prepared previously and the reaction mixture lasted for 8 h. At the end, the reaction solution was filtered. The protein content of the remained meals on the filter membrane and the free amino acids in the filtered solution were measured. The in vitro digestibility was calculated based on the protein loss in the five protein sources and the production of free amino acids after digestion as previously described by Wang et al. (2009a, b).

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Data were tested for normality using Shapiro-Wilk test. All measured variables were normally distributed except MDA concentration, GSH-Px activity, and protein digestibility. Means of each normal distributed measured variable were analyzed by using one-way analysis of variance (ANOVA) test. Duncan's multiple range test was used to rank the groups whenever differences were obtained. The differences in MDA concentration, GSH-Px activity, and protein digestibility for various plant protein sources were examined by using Kruskal-Wallis test followed by Mann-Whitney test for specific comparisons whenever differences were obtained. Significant differences of each variable measured were detected at $p < 0.05$. All statistical analyses were performed using SPSS 23 software (SPSS Inc., Chicago, IL, USA).

Results

Growth parameters, survival rate, feed efficiency, hepatosomatic index, and body composition

The results on growth performance parameters, survival, feed efficiency, hepatosomatic index, and body composition are shown in Fig. 1. The different sources of plant-based proteins affected significantly the weight gain of prawns ($p = 0.021$). Prawns fed the diets containing SBM (730.96 ± 33.50%) and CSM (672.32 ± 74.52%) obtained higher weight gain than those fed on RPM (503.71 ± 50.85%) and PNM (507.79 ± 52.10%) diets ($p < 0.05$). However, the weight gain for prawns fed on diets SBM and CSM was statistically similar to those fed on FM (595.92 ± 23.95%) diet ($p = 0.090$). Likewise, prawns fed on FM diet had statistically comparable weight gain to those fed on RPM and PNM diets ($p = 0.236$).

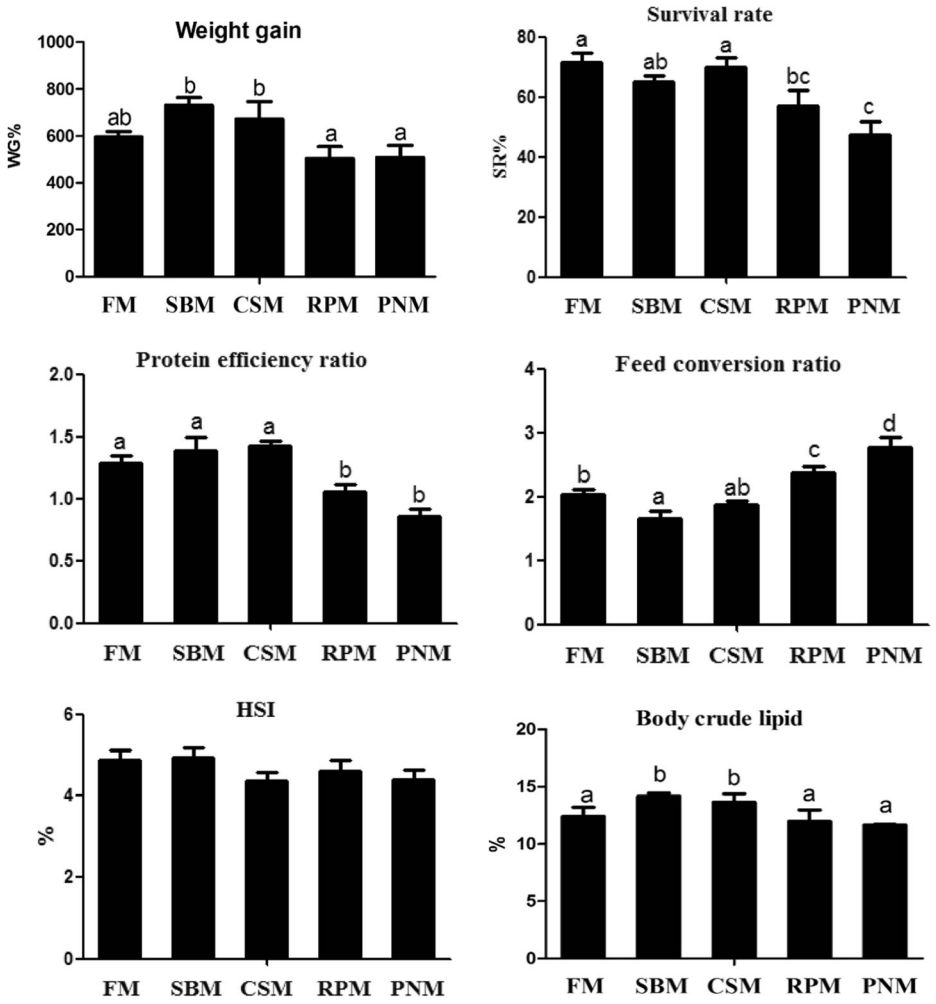


Fig. 1 Effects of different protein sources on weight gain (WG), survival rate (SR), feed conversation rate (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) in the juvenile *Macrobrachium nipponense*. Vertical bars show SE. Values with different letters on each column are statistically different at $p < 0.05$

The survival rate of the prawns fed the different protein sources was significantly different ($p = 0.002$). The survival rate of prawns fed on FM (71.35 ± 3.47%) and CSM (70.00 ± 2.97%) diets was significantly higher compared to those fed on RPM (57.00 ± 5.32%) and PNM (47.50 ± 4.17%) diets ($p < 0.05$). Similarly, prawns fed on SBM diet (65.06 ± 2.06%) had higher survival rate than those fed on PNM diet ($p < 0.05$). Statistically similar survival rates were obtained for prawns fed on FM, SBM, and CSM diets ($p = 0.280$), RPM and SBM ($p = 0.151$) diets, and RPM and PNM diets ($p = 0.094$). In this experiment, the prawns died mostly in the earlier days. This might be mainly because of the combat between prawns rather than the nutrient effects of different diets.

Feeding prawns by using the different protein sources diets affected significantly the protein efficiency ratio (PER) of the prawns ($p = 0.000$). The PER of prawns fed on FM (1.29 ±

0.06%), SBM (1.38 ± 0.11%), and CSM (1.43 ± 0.03%) diets was significantly higher than those fed on RPM (1.05 ± 0.06%) and PNM (0.85 ± 0.06%) diets ($p < 0.05$). Statistically similar PER was obtained for prawns fed on FM, SBM, and CSM diets ($p = 0.187$), and RPM and PNM diets ($p = 0.063$).

The feed conversion rate (FCR) of the prawns fed the different protein sources was significantly different ($p = 0$). The FCR of prawns fed on SBM (1.66 ± 0.12%), CSM (1.88 ± 0.06%), and FM (2.03 ± 0.08%) diets was significantly lower than those fed on RPM (2.37 ± 0.10%) and PNM (2.77 ± 0.16%) diets ($p < 0.05$). FCR of prawns fed on SBM was significantly lower than those fed on FM diet ($p < 0.05$). Statistically similar FCRs were obtained for prawns fed on SBM and CSM diets ($p = 0.180$) and FM and CSM diets ($p = 0.329$). Feeding prawns with the four plant protein sources did not affect the hepatosomatic index ($p = 0.355$; Fig. 1).

The body lipid content of the prawns was affected significantly by the different sources of proteins ($p = 0.001$). The body lipid content of the prawns fed on SBM (14.17 ± 0.30%) and CSM (13.66 ± 0.47%) diets was significantly higher than those fed on RPM (11.98 ± 1.55%) and PNM (11.65 ± 0.05%) diets ($p < 0.05$). Statistically similar crude body lipid of prawns was obtained for prawns fed on SBM and CSM diets ($p = 0.406$) and those fed on FM (12.40 ± 1.24%), RPM, and PNM diets ($p = 0.252$).

Histological observation, oxidative stress, and total hemocyte count

No obvious histological characteristic alterations were observed by light microscopy neither in hepatopancreas nor intestines (Supplementary Fig. S1) among the five treatments. Moreover, the five protein sources in the present study did not significantly affect the activity of GSH-Px ($p = 0.327$), hepatic MDA ($p = 0.115$), SOD content, and total hemocyte number ($p > 0.05$; Fig. 2).

The amino acid composition of experimental diets and prawn muscle

The total amino acids of the five formulated diets used in the feeding experiment are given in Table 2. The methionine content in the four plant protein meals was relatively lower than that of the fishmeal diet. Furthermore, cysteine content in fishmeal diet was lower than the other four groups. Comparably, the amino acid composition of the muscle of the experimental prawn from different treatments is provided in Table 3. The composition of most of the amino acids tested was approximately the same, with some minor differences. The cysteine content in muscle was 0.52 ± 0.19% for prawns fed on FM diet 0.10 ± 0.11% for those fed on SBM diet and 0.21 ± 0.37% in the prawns fed on CSM diet. The different sources of proteins affected significantly the cysteine content in muscle ($p = 0.001$). Cysteine content was significantly higher for the *M. nipponense* fed on RPM diet than those fed on SBM diet ($p = 0.033$). However, there was no statistical differences in *M. nipponense* cysteine content among the other four experimental groups ($p = 0.057$).

In vitro digestion rate of the five protein sources

We measured the in vitro digestibility in the present study. The digestibility of dry matter was comparable among groups ($p > 0.05$; Fig. 3). The protein digestibility was different among the various sources of proteins ($p = 0.029$). The protein digestibility was significantly higher for

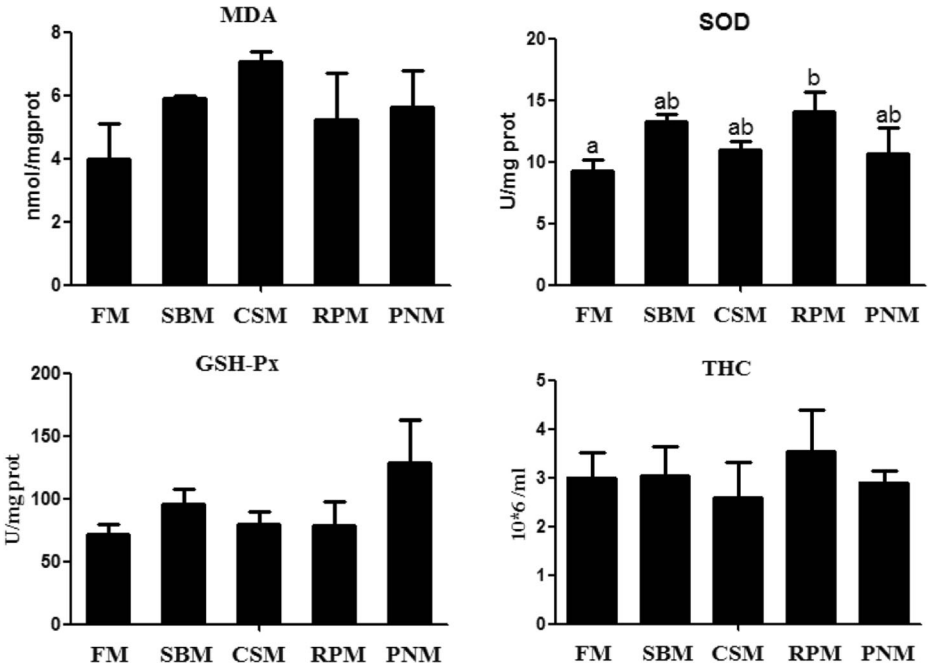


Fig. 2 Effects of different protein sources on the antioxidant enzyme activities (SOD, GSH-Px), malondialdehyde (MDA) content of hepatopancreas, and total hemocyte count (THC) in hemolymph of the juvenile *Macrobrachium nipponense*. Vertical bars show SE. Values with different letters on each column are statistically different at $p < 0.05$

prawns fed on SBM diet (43.67 ± 4.24%) than those fed on FM (34.46 ± 0.24%) ($p = 0.05$), CSM (31.87 ± 0.52%) ($p = 0.05$), and PNM (32.55 ± 2.15%) ($p = 0.05$). However, prawns fed on SBM diet had no significant differences in protein digestibility compared with those fed on RPM (36.63 ± 0.90%) ($p = 0.127$). Moreover, the prawns fed on FM diet had significantly higher protein digestibility than those fed on CSM ($p = 0.05$) and RPM ($p = 0.05$) diets, but prawns fed on FM diet had no significant difference with those fed on PNM ($p = 0.513$). Furthermore, the prawns fed on CSM had significantly lower protein digestibility than those fed on RPM ($p = 0.05$), but CSM diet had statistically comparable protein digestibility to prawns fed on PNM ($p = 0.827$). The prawns fed on RPM and PNM had statistically comparable protein digestibility ($p = 0.127$).

Discussion

The effect of fishmeal replacement with plant protein on growth and feed utilization

The main purpose of the present experiment was to investigate the feasibility of fishmeal partial replacement by using common plant protein sources. Replacing fishmeal with plant protein sources has been shown to result in reduced growth performance, survival rate, feed intake, and protein efficiency in some fish studies (Kaushik et al. 1995, Gómez-Requeni et al. 2004; Sitjà-Bobadilla et al. 2005; Zheng et al. 2012). Indeed, some of the plant protein sources (RPM and PNM) used in the present study also affected growth performance. In general,

Table 2 Amino acid composition of the test diets (% in dry matter)

Amino acids	Test diets				
	FM	SBM	CSM	RPM	PNM
EAA					
Arg	1.97	2.13	2.57	1.97	2.50
His	0.79	0.84	0.81	0.80	0.79
Ile	1.39	1.4	1.26	1.34	1.33
Leu	2.42	2.46	2.23	2.34	2.38
Lys	2.34	2.23	2.04	2.17	2.05
Met	0.98	0.83	0.82	0.86	0.79
Phe	1.36	1.5	1.46	1.35	1.45
Thr	1.36	1.36	1.26	1.35	1.28
Val	1.57	1.58	1.51	1.57	1.54
∑EAA	14.18	14.33	13.96	13.75	14.11
NEAA					
Ala	2.21	2.02	1.96	1.99	2.02
Asp	2.97	3.22	2.97	2.75	3.27
Cys	0.19	0.24	0.24	0.33	0.21
Glu	4.61	5.08	5.16	4.80	5.19
Gly	2.59	2.34	2.3	2.34	2.51
Pro	1.48	1.68	1.53	1.68	1.61
Ser	1.31	1.42	1.33	1.29	1.42
Tyr	1.05	1.13	1.02	1.04	1.12
∑NEAA	16.41	17.13	16.51	16.22	17.35
∑EAA/∑NEAA	0.86	0.84	0.85	0.85	0.81

EAA essential amino acid, *NEAA* nonessential amino acid

among the growth and feed utilization indexes, the WG, FCR, and PER in the prawns fed the SBM and CSM were better than those fed on FM and had significant differences compared to those fed on RPM and PNM. Prawns fed the SBM diet had higher WG and lower FCR than CSM. Based on growth performance, survival rate, and feed efficiency, SBM and CSM are potential plant protein sources for fishmeal partial replacement in *M. nipponense*. Results indicated that RPM and PNM may not be proper plant protein sources for replacing 25% fishmeal in *M. nipponense* because they significantly impaired growth performance.

Soybean meal is an important alternative protein source which has been widely used in aquaculture feed industry. A number of studies have proved that a proper replacement of fishmeal with SBM does not affect growth performance and feed utilization in fish and crustaceans (Robaina et al. 1995, 1998; Kissil et al. 2000; Pereira and Oliva- Teles, 2002; Ding et al. 2015a, b). Compared to SBM, the application of CSM in fish diets is controversial. Robinson (1991) reported that up to 100% of SBM could be replaced with CSM if enough lysine was supplemented in feed for channel catfish. Fowler (1980) found that up to 34% CSM can be used to replace dietary SBM without growth depression in two Pacific salmon species. However, some studies have indicated that replacing fishmeal by CSM produces worse effects than other plant protein sources because the gossypol in the cottonseed might impair reproduction of fish (Dabrowski et al. 2001; Wang et al. 2014). Interestingly, CSM has been reported to have good effects in replacing fishmeal in diets for crustaceans. A previous study conducted by Jiang et al. (2012) in Chinese mitten crab, *Eriocheir sinensis*, reported that fishmeal can be replaced by an equal percentage of CSM and SBM resulting in similar growth performance. Lim (1996) found that the growth performance of *Litopenaeus vannamei* was not

Table 3 Amino acid composition in the muscle of the *M. nipposence* fed with different diets (% in dry matter)

Amino acids	Test diets				
	FM	SBM	CSM	RPM	PNM
EAA					
Arg	7.37 ± 0.19^{a}	7.44 ± 0.06^{ab}	7.80 ± 0.16^{b}	7.68 ± 0.05^{b}	7.59 ± 0.14^{ab}
His	2.24 ± 0.17	2.54 ± 0.04	2.57 ± 0.36	2.31 ± 0.22	2.53 ± 0.06
Ile	3.64 ± 0.09	3.65 ± 0.02	3.72 ± 0.10	3.75 ± 0.04	3.64 ± 0.05
Leu	5.98 ± 0.15^{ab}	5.99 ± 0.04^{ab}	6.09 ± 0.12^{ab}	6.16 ± 0.07^{b}	5.94 ± 0.11^{a}
Lys	6.67 ± 0.18^{a}	6.67 ± 0.05^{a}	6.84 ± 0.14^{ab}	6.94 ± 0.07^{b}	6.66 ± 0.12^{a}
Met	2.22 ± 0.07^{ab}	2.08 ± 0.11^{a}	2.17 ± 0.06^{ab}	2.31 ± 0.03^{b}	2.11 ± 0.11^{a}
Phe	3.35 ± 0.09^{a}	3.31 ± 0.02^{ab}	3.35 ± 0.08^{a}	3.36 ± 0.04^{a}	3.22 ± 0.06^{b}
Thr	2.89 ± 0.08	2.88 ± 0.02	2.91 ± 0.05	2.98 ± 0.03	2.94 ± 0.06
Val	3.48 ± 0.09^{a}	3.48 ± 0.02^{a}	3.59 ± 0.09^{ab}	3.64 ± 0.02^{b}	3.58 ± 0.06^{ab}
∑EAA	37.85 ± 0.93	38.06 ± 0.35	38.96 ± 1.01	39.13 ± 0.13	38.21 ± 0.62
NEAA					
Ala	4.60 ± 0.11^{b}	4.84 ± 0.01^{a}	4.76 ± 0.11^{ab}	4.75 ± 0.12^{ab}	4.28 ± 0.08^{c}
Asp	8.57 ± 0.23	8.50 ± 0.06	8.65 ± 0.17	8.73 ± 0.11	8.47 ± 0.15
Cys	*0.52 ± 0.19*^{a}	*0.10 ± 0.11*^{a}	*0.21 ± 0.37*^{a}	*0.56 ± 0.22*^{b}	*0.20 ± 0.13*^{a}
Glu	12.46 ± 0.36	12.41 ± 0.05	12.77 ± 0.25	12.60 ± 0.14	12.61 ± 0.25
Gly	5.71 ± 0.15^{a}	5.73 ± 0.03^{a}	5.49 ± 0.12^{a}	5.09 ± 0.27^{b}	4.24 ± 0.07^{c}
Pro	3.07 ± 0.07^{b}	2.82 ± 0.03^{a}	3.04 ± 0.07^{b}	3.10 ± 0.02^{b}	3.41 ± 0.05^{c}
Ser	3.01 ± 0.08^{a}	3.02 ± 0.02^{a}	3.04 ± 0.04^{ab}	3.13 ± 0.03^{b}	3.09 ± 0.07^{ab}
Tyr	2.99 ± 0.08^{a}	2.96 ± 0.02^{ab}	3.00 ± 0.06^{a}	3.00 ± 0.04^{a}	2.87 ± 0.07^{b}
∑NEAA	40.95 ± 1.20^{a}	40.38 ± 0.31^{ab}	40.97 ± 0.51^{a}	41.19 ± 0.91^{a}	39.18 ± 0.75^{b}
∑EAA/∑NEAA	0.92 ± 0.01^{a}	0.94 ± 0.00^{ab}	0.95 ± 0.02^{b}	0.95 ± 0.02^{b}	0.98 ± 0.00^{c}

affected by feeding them with diets containing less than 26.5% CSM. In the present study, we also confirmed that SBM and CSM had similar fishmeal-replacing effects, and would not cause adverse effects when the replacing ratio is not more than 25%.

The effect of fishmeal replacement with plant protein sources on oxidative stress and hepatosomatic index

The replacement of fishmeal with plant protein sources has been shown to result in some pathological consequences in fish, such as fatty liver and higher oxidative stress (Sitjà-Bobadilla et al. 2005). Some studies have reported that excess plant protein sources would

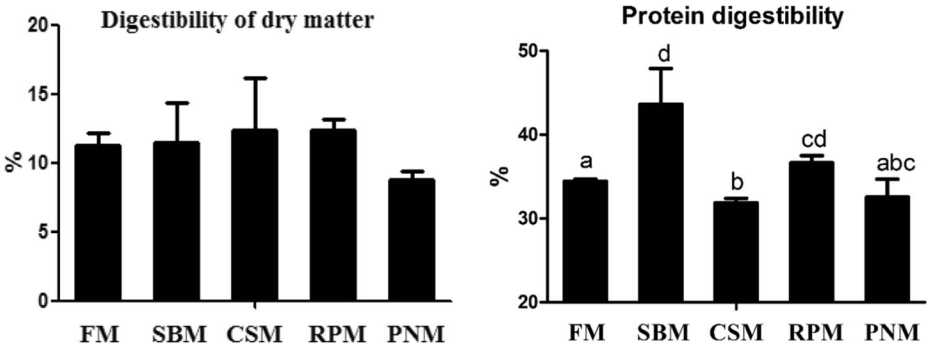


Fig. 3 The in vitro digestibility of five protein sources. Vertical bars show SE. Values with different letters on each column are statistically different at $p < 0.05$

induce oxidative stress and decrease immune activity in aquatic animals (Liu et al. 2012; Wang et al. 2014). In this study, the five protein sources in the present study did not significantly affect the activities of GSH-Px and SOD as well as the hepatic MDA content. MDA is an indicator of endogenous oxidative damage and a final product of lipid peroxidation. The existence of statistical similar levels of MDA among *M. nipponense* fed the five diets suggests there was a balance between reactive oxygen species (ROS) production and antioxidant capacity in the liver of the test prawns. This indicates that the growth promoting and depressing effects of SBM, CSM, and RPM, PNM, respectively, did not primarily cause oxidative stress in the present study. Furthermore, the relative weight of hepatopancreas was comparable in all groups and the feed intake was also not affected by the fishmeal partial replacement using the four plant protein sources. Moreover, the histological analysis of hepatopancreas and intestines showed no significant differences among *M. nipponense* fed the different protein sources. These results indicate that the 25% plant meal contents did not induce any obvious damage to *M. nipponense,* at least in the 8 weeks of the trial.

The effect of fishmeal replacement with plant protein sources on muscle AA components

The unbalanced amino acid composition in most plant protein sources commonly causes low utilization of plant protein in aquatic animals, especially in carnivorous species (Cho and Kaushik 1990; Hajen et al. 1993; Gomes et al. 1995). Different studies have shown variable results on amino acid composition in aquatic species, which might be related to the experimental methods and conditions, the growth stage of aquatic animals and feed sources, especially the essential amino acids contents (Petzke et al. 1997; Gatlin et al. 2007; Li et al. 2011). Currently, limited systematic research has been conducted on the essential amino acid requirements and limiting effects of various amino acids on different life stages of *M. nipponense*.

In the present study, neither in the diets nor in the muscle, the amino acid composition among *M. nipponense* groups was generally comparable. Contents of the essential amino acids (EAA) are generally lower in SBM and some other plant meals than in fishmeal (Gatlin et al. 2007). The total EAA contents in the FM, SBM, and PNM diets were a slightly higher than that in CSM and RPM diets in this experiment. The EAA contents in muscle of FM, SBM, and PNM feeding groups were about the same, meanwhile slightly higher in CSM and RPM groups. Notable, the contents of cysteine in muscle of *M. nipponense* fed on SBM, CSM, and PNM groups were obviously lower than those fed on fishmeal. Among the triplicate tests of total amino acids, cysteine could not be detected in *M. nipponense* fed on SBM and CSM groups after repeated analysis. Cysteine is usually an exception to this observation, as it was also verified by its higher concentrations in SBM and the three other plant meals in our experiment. As noted across multiple aquaculture species, cysteine can meet about 40 to 60% of the total sulfur amino acid (TSAA; methionine plus cysteine) requirement. Supplemental methionine, as one of the limiting essential amino acids, can overcome part of the growth reduction observed when a large percentage of the total dietary protein is from soy origin (Wilson 2002). To our knowledge, cysteine is the crucial limiting amino acid for intracellular GSH synthesis. Glutathione is a vital molecule for antioxidant reactions and is synthesized from cysteine. In the present study, the plant protein sources added diets contained more cysteine than full fishmeal, meanwhile, prawns muscle fed with SBM and CSM showed relatively lower cysteine contents. This implies that feeding prawns with plant protein dietary

might need more cysteine in order to adapt to the changing amino acid component intake, especially in SBM and CSM groups. The underlying connection between the variations in cysteine or/and other amino acids and physiological as well as nutritional status need further considerable and precise research.

However, under the conditions of the present experiment, it seems that the slight differences in few amino acids among the different plant protein groups were unlikely to be the main reason that caused significant differences in growth and feed utilization among the different prawn groups. This is because fishmeal protein was only partial (25%) replaced by the plant proteins sources. The inclusion of 75% fishmeal as a protein source still could provide the relative stability of the amino acid profile among groups. This finding also indicates that the mixture of cottonseed and soybean meal with methionine and cysteine supplementation might provide optimum growth, survival, and feed utilization in diets for *M. nipponense*. There has not been any systematic study to explore the direct interactions between methionine and cysteine. The reasonable portion of cysteine in sulfur amino acids might contribute a significant amount.

In vitro digestibility and anti-nutritional factors of the four plant protein sources

In the absence of a single meal analogue having some amino acid balance suitable to serve as sole protein source especially for cultured *M. nipponense*, it is important to evaluate the nutritional value of alternative ingredients and formulate diets based on a mixture of such components which can collectively replace fishmeal. Consequently, reliable data on the bioavailablity of nutrients and digestibility of different protein sources for *M. nipponense* should be considered as a necessary step in formulating practical diets.

Poor digestibility also contributes to the relatively low utilization of many plant protein sources in aquatic animals (Robaina et al. 1997; Jiang et al. 2012). The in vitro activities of enzyme extracted from digestive system could mimic and reflect the digestibility of feed in vivo. Soybean meal showed the highest digestibility in the in vitro assay. This result accords with the higher growth performance of *M. nipponense* in the SBM group. However, it could not explain the significant difference in growth performance between prawns fed on CSM and RPM/PNM diets.

There is a close relationship between animal nutrition and body immunity, which was depicted by similar trends in WG and SR, respectively, in the present study. Anti-nutritional factors in plant protein sources, including lectins in soybeans, erucic acid, and glucosinolates in rapeseed and gossypol in cottonseed, have been commonly regarded as the remarkable reasons to adversely affect the utilization of plant protein sources in aquatic animals (Gatlin et al. 2007). Previous studies have demonstrated that reduced digestive enzyme activities and nutrient digestibility, coupled with the high potential toxicity of mycotoxins in PNM, limit its application in feed industry (Liu et al. 2012). In the present study, the decreased growth and feed efficiency in prawns fed the PNM and RPM diets might be caused by the existence of anti-nutritional factors, but this observation needs further investigation for confirmation.

In conclusion, our study illustrates that soybean and cottonseed meals could be good plant protein sources to partial replace at least 25% fishmeal protein in diets of juvenile *M. nipponense* based on growth performance, feed utilization, survival, and whole-body compositions. Meanwhile, rapeseed and peanut meals showed non-negligible negative effects at the same replacing level. These results indicate that it is practicable to partial replace 25% fishmeal with alternative plant proteins for *M. nipponense*. Further work is necessary in order to find more other alternative supplement sources to balance amino acids and to restrain anti-nutritional factors or a cocktail mixture of meals with different proportions.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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