



# Partial or Total Replacement of Soybean Meal by Cottonseed Meal in Practical Diets for Chinese Mitten Crab, *Eriocheir sinensis*: Effects on Oxygen Consumption, Ammonia Excretion, O:N Ratio and Amino Transferases Activities

HaiBo Jiang<sup>1</sup>, LiQiao Chen<sup>1,\*</sup>, ErChao Li<sup>1</sup>, XueQin Jiang<sup>1</sup>, ShengMing Sun<sup>1</sup>

<sup>1</sup> East China Normal University, School of Life Science, Shanghai 200062, China.

\* Corresponding Author: Tel.: +86.021 62233637; Fax: +86.021 62233637;  
E-mail: lqchen@bio.ecnu.edu.cn

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## Abstract

This study investigate the effect of replacement of soybean meal (SBM) by cottonseed meal (CSM) on oxygen consumption, ammonia nitrogen excretion, O:N ratio and aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities for *Eriocheir sinensis*. Seven isocaloric and isonitrogenous diets were tested with five replicates. CSM replaced 0, 33, 66 and 100% of SBM in four diets (CSM0, CSM33, CSM66 and CSM100) and lysine was added to other three diets (CSM33+lys, CSM66+lys and CSM100+lys). Oxygen consumption rates of crab fed CSM66+lys and CSM100+lys were significantly lower than crab fed CSM0, and crab fed CSM100+lys was significantly lower than crab fed CSM33 and CSM66. The ammonia excretion of crab in the CSM0 was significantly higher than that in all other treatments. Crab fed CSM33+lys, CSM66+lys and CSM100+lys discharged less ammonia than crab fed CSM33, CSM66 and CSM100. The O:N ratio indicated that protein was decreasingly used as an energy substrate when SBM was replaced by CSM. With lysine supplementation, the crab made use of lipid and carbohydrates as the most important sources of energy metabolism. Activities of ASAT in CSM66+lys and CSM100+lys were significantly lower than that in CSM0, CSM33 and CSM100. Results suggest that 100% of SBM can be replaced by CSM with lysine supplement in crab without affecting growth performance and with decreasing level of ammonia released into the water.

**Keywords:** *Eriocheir sinensis*, soybean meal, cottonseed meal, oxygen consumption, ammonia excretion.

## Introduction

Protein is basis for aquaculture animals, and feeding protein costs make up a large percentage of the total expenses of aquafeeds. Soybean meal (SBM) has been the most common source of plant protein as a fish meal replacer in aquaculture feed because of its high protein content, relatively well-balanced amino acid profiles, reasonable price and steady supply (EI-Sayed, 1999). However, high demand of SBM has made it a relatively expensive protein source after fish meal. Therefore, the search for alternative plant proteins to replace soybean meal has been gained increasing interest in the research of aquaculture nutrition.

Cottonseed meal (CSM) is a source of plant protein that has long been used to feed terrestrial and aquatic animals (Li and Robinson, 2006; Gatlin III *et al.*, 2007; Lim and Lee, 2008). Cottonseed meal is the third largest oil-seed meal product in world production after soybean meal and rapeseed meal (Lee *et al.*, 2006) and it is a rich source of arginine, an

essential amino acid for aquatic animals, which is higher than that in fish meal and soybean meal. The amount of CSM that can be included in aquaculture diets depends mainly on the species and levels of gossypol and available lysine (EI-Saidy *et al.*, 2004). Because gossypol binds to lysine leading to inactivate its biological availability, supplemental lysine must be added if the level of CSM inclusion is high (Robinson and Li., 2008). Several studies on the utilization of CSM as substitute for FM and SBM in rainbow trout (*Oncorhynchus mykiss*) (Rinhard *et al.*, 2003; Lee *et al.*, 2006), channel catfish (*Ictalurus punctatus*) (Robinson and Li, 1994; Robinson and Li, 2008), hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) (Yue and Zhou, 2008), Nile tilapia (*Oreochromis niloticus*) (EI-Saidy and Saad, 2011), parrot fish (*Oplegnathus fasciatus*) (Lim and Lee, 2009), and white shrimp (*Penaeus vannamei*) (Lim, 1996) diets have been conducted on growth performance, physiological function and reproductive performance during the last two decades. A complete evaluation of new formula feeds for aquatic animals include not

only their nutritional value, but also their utilization by animals. Specifically new combinations of protein sources are of particular interest, since unbalanced dietary amino acid contents could result in an increased deamination, therefore increase level of ammonia released into the water (Robaina *et al.*, 1997). While the level of ammonia in water is at or above certain standard, it is contaminated to water environment and toxic to aquatic animals. Alanine aminotransferase (ASAT) and aspartate aminotransferase (ALAT) are two enzymes, which are important in transamination of amino acids (Hansen *et al.*, 2007).

Oxygen consumption is an important parameter to describe the respiratory capacity and estimate metabolic expenditure of animals to maintain their vital functions through an aerobic metabolism (Montagna and Collins, 2008; Valverde *et al.*, 2009). It is influenced on the one hand, by environmental factors such as oxygen concentration (McMahon, 2001), temperature (Perera *et al.*, 2007) or salinity (Li *et al.*, 2007), and by internal factors such as body weight (Carvalho and Pan, 1997), moulting cycle (Penkoff and Thurberg, 1982), feeding state (Perera *et al.*, 2007), on the other. Apart from that, the ingredients of diet fed could cause significant changes in oxygen consumption (McKenzie *et al.*, 1997; Grisdale-Helland *et al.*, 2002; Du and Niu, 2003). The parameter oxygen uptake: ammonia excretion (O:N) is a good indicator of energy metabolic substrates (carbohydrate, lipid and protein) (Montagna and Collins, 2008). The pure protein catabolism will yield O:N ratios in the range 3 to 16, protein and some lipid will lead to O:N ratios from 17 to 49, equal amounts of lipid and protein will correspond to values between 50 and 60, while higher values indicates that energy metabolic substrates are lipid and carbohydrates (Mayzaud and Conover, 1988; Perera *et al.*, 2005).

The Chinese mitten crab (*Eriocheir sinensis*) is a popular delicacy in China, Japan and other Asian countries. Recently, significant development has been made on farming technology of this species and its production has reached half million metric tons with a \$1.25 billion revenue in China (Yang and Zhang, 2005; Herborg *et al.*, 2005). However, its profit has encountered a great challenge because feed has accounted for large proportion of the total production cost (Muzinic *et al.*, 2006). Recent study have confirmed that 66% of SBM could be replaced with CSM in the diets of Chinese mitten crab without compromising growth, feed utilization, and body composition, and total replacement of SBM with CSM would be possible when the diets are supplemented with lysine during a 10-week trial (Jiang *et al.*, 2012).

The purposes of the present study was conducted to compare the ammonia excretion, oxygen consumption, O:N ratios and amino transferases activities of juvenile *E. sinensis* fed diets containing progressively increasing levels of CSM at the expense

of SBM with or without supplemental microcapsule lysine.

## Materials and Methods

### Experimental Diets

Seven isonitrogenous (approximately 36% crude protein) diets with isocaloric value ( $12.3 \text{ kJ g}^{-1}$ ) were formulated to replace 0 (control), 33%, 66%, and 100% of SBM protein by a corresponding amount of protein with CSM to form four diets (CSM0, CSM33, CSM66, and CSM100) and other three diets with supplementation of microcapsule lysine-HCl (CSM33+lys, CSM66+lys, and CSM100+lys, respectively). All diets were formulated to be isonitrogenous and isoenergetic (Table 1). Diets were prepared by mixing dry ingredients, and then oil and water were added (40%, v/w) to form a soft dough. The ingredients were mixed to facilitate pelleting by bibolt plodder (TS 12, East China Sea Fisheries Research Institute, Shanghai, China). The pellets (1.0 mm diameter) were extruded and air-dried to <10% moisture before being stored at  $-20^{\circ}\text{C}$ .

### Experimental Animals and Feeding Trial

Juveniles of Chinese mitten crab *E. sinensis* were obtained from Chongming Island Fisheries (Shanghai, China), and were stocked in fifty polyvinyl tanks (300 L) with 30 crabs each. Sixteen PVC tubes (50 mm in diameter  $\times$  12 cm long) and 10 tiles (15  $\times$  20 cm) were placed in each tank to reduce animal aggression. Crabs were acclimatized for 1 week and then the crabs ( $1.62 \pm 0.089 \text{ g}$  mean  $\pm$ SD) were randomly stocked into thirty five 300 L polyvinyl tanks at a density of 20 crabs per tank. Each tank with 20cm deep water was covered with a vinyl sheet to prevent crabs from crawling out. Crabs in five replicate tanks were randomly assigned to each of the experimental diets and were fed twice daily at 0800-0900 h and 1700-1800 h with 8-10% wet body weight per day for 6 weeks. Crab weight was measured every 3 weeks for feed adjustments. Excess feed on the tank bottom was siphoned out 2 h after feeding, exuviae and dead crabs were checked daily.

Day length (approximately 11h light /13h dark) and water temperature ( $17\text{-}25^{\circ}\text{C}$ ) followed natural changes, dissolved oxygen was above  $6.5 \text{ mg L}^{-1}$ , ammonia nitrogen was between  $0.01 \text{ mg L}^{-1}$  and  $0.03 \text{ mg L}^{-1}$ , pH value was 7.9-8.4.

### Sampling

All crabs were weighted individually at start and at the end of the experiment (6 weeks). Following 24h fasting, 10 crabs per dietary treatment were withdrawn for hepatopancreas samplings. Hepatopancreas were rapidly excised, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until analysis.

**Table 1** Formulation and proximate analysis of the experimental diets

Ingredients (g / 100 g diet)	Experimental diets						
	CSM0	CSM33	CSM66	CSM100	CSM33+lys	CSM66+lys	CSM100+lys
Fish meal	20	20	20	20	20	20	20
Soybean meal	60	40	20	0	40	20	0
Cottonseed meal	0	21	43	64	21	43	64
Lysine-HCl <sup>a</sup> (39%)	0	0	0	0	0.33	0.65	0.97
Corn starch	2.5	1.5	1.5	0.5	1.5	1.0	0
Fish oil	1.8	2.0	2.0	2.2	2.0	2.0	2.2
Soybean oil	1.8	2.0	2.0	2.2	2.0	2.0	2.2
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix <sup>b</sup>	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mix <sup>c</sup>	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose	5.40	5.00	3.00	2.60	4.67	2.85	2.13
Binder <sup>d</sup>	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Proximate composition (g / 100 g diet)							
Dry matter	90.29	91.60	91.70	91.43	91.75	91.11	91.81
Protein	36.21	36.24	35.68	36.12	36.47	36.10	36.98
Lipid	6.88	7.02	6.79	6.93	7.08	6.83	6.81
Ash	11.80	12.35	12.61	12.53	11.77	12.46	11.78
Crude fibre	13.42	14.60	14.53	14.53	15.04	13.63	14.74
Gross energy (kJg <sup>-1</sup> ) <sup>e</sup>	12.30	12.25	12.33	12.28	12.27	12.29	12.26
Lysine	2.39	2.26	2.14	2.01	2.39	2.39	2.39

<sup>a</sup> Microcapsule Lysine-HCl (Hinapharm Pharmaceutical Co. Ltd, Guangdong, China)

<sup>b</sup> Mineral mixture(%): NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 13.00; KH<sub>2</sub>PO<sub>4</sub>, 21.50; Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O, 24.733; CaCO<sub>3</sub>, 14.665; Ca-lactate, 16.5; MgSO<sub>4</sub>, 4.88; AlCl<sub>3</sub>, 0.945; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.511; Fe-citrate, 0.061; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.108; KI, 0.058; CuCl<sub>2</sub>·2H<sub>2</sub>O, 0.0647; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.176; KCl, 2.8

<sup>c</sup> Vitamin mixture (Minshen Bio-tech, Co. Ltd, Hangzhou, China), per 100 mix contained: vitamin A, 420,000 IU; vitamin C, 6,000 mg; vitamin E, 2,000 mg; cholecalciferol, 120,000 IU; phytanadione, 1,000 mg; thiamine, 1,000 mg; riboflavin, 1,000 mg; pyridoxine, 1,600 mg; cobalamin, 2 mg; niacin, 5,000 mg; folic acid, 400 mg; inositol, 6,000 mg; biotin, 10 mg; calcium pantothenate, 3,500 mg;

<sup>d</sup> Sodium carboxymethyl cellulose (Shanghai Chemical, Shanghai, China)

<sup>e</sup> Gross energy was calculated based on protein=16.7kJg<sup>-1</sup>; lipid=37.6kJg<sup>-1</sup>; NFE=16.7kJg<sup>-1</sup> (Ai *et al.*, 2004).

## Diets Composition Analyses

Diet samples were analyzed as follows: dry matter was desiccated in an oven at 105°C for 24 h, and crude protein was determined by measuring nitrogen (N× 6.25) using the Kjeldahl method. Crude lipid was determined by ether extraction using Soxhlet and ash was by combustion at 550°C (AOAC 1995). Crude fiber by the fritted glass crucible method after treated with H<sub>2</sub>SO<sub>4</sub> and NaOH. Nitrogen-free extract was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fiber and ash from the original amount (i.e., 100%).

## Growth Parameters

Growth performance was determined as follows:

Weight gain (WG, %) = 100 × (final body weight - initial body weight)/initial body weight;

Specific growth rate (SGR) = 100 × (ln final weight - ln initial weight)/days of the experiment;

## Oxygen Uptake and Ammonia Excretion

Each sealed respiratory chamber (2500 ml) allowed oxygen consumption and ammonia excretion to be measured for 2 crab (3.37±0.29g mean ± SD)

selected from each diet, filled up with oxygen-saturated water. Three replications were used in each treatment and control. Dissolved oxygen (DO) and ammonia-N nitrogen were measured about 24h. Oxygen concentration was measured using HQ30D DO meter (HACH, Germany). Ammonia excretion was determined using ammonia-N nitrogen analysis kit (Palintest, UK). DO and ammonia excretion at the end of the experiment were expressed as mg O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> and µg NH<sub>4</sub>-N g<sup>-1</sup> h<sup>-1</sup>.

Oxygen consumption rate and ammonia excretion rate were calculated as following:

$$M_{O_2} = V \times (X_1 - X_2) / (t \times W)$$

$$M_{NH_4-N} = 1000 \times V \times (X_3 - X_4) / (t \times W)$$

$M_{O_2}$  is the oxygen consumption rate (mg O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>), V is the volume (L) of the chambers, X<sub>1</sub> is the oxygen concentration (mg O<sub>2</sub> L<sup>-1</sup>) in a blank control chamber finally, X<sub>2</sub> is the oxygen concentration (mg O<sub>2</sub> L<sup>-1</sup>) in the chamber finally,  $M_{NH_4-N}$  is the ammonia excretion rate (µg NH<sub>4</sub>-N g<sup>-1</sup> h<sup>-1</sup>), X<sub>3</sub> is the ammonia-N concentration (mg NH<sub>4</sub>-N L<sup>-1</sup>) in the chamber finally, X<sub>4</sub> is the ammonia-N concentration (mg NH<sub>4</sub>-N L<sup>-1</sup>) in a blank control chamber finally, t is the time (24h), W (g) is the body weight of the experimental crab.

O:N ratio, the index of metabolism is expressed

by the ratio of oxygen consumption and ammonia excretion rates by atoms.

### Enzyme Activities

Crude extracts of hepatopancreas for assaying enzyme activities were obtained by homogenization of frozen tissue in 100 volumes of ice-cold buffer (PBS, pH 7.0), and the activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined with commercially available kit (Jiancheng, Nanjing, China)

### Statistical Analysis

Results are expressed as means  $\pm$  standard deviation. All data were compared using a one-way analysis of variance (ANOVA), and the significance of mean differences was compared using the Duncan's multiple range test. All statistical analyses were performed using SPSS11.5 and the probability of significant differences was set at  $P < 0.05$ .

### Results

Crab growth performance and survival data presents in Table 2. Crab survival, final body weight, weight gain and specific growth rate were not different among dietary groups ( $P > 0.05$ ).

Oxygen consumption, ammonia excretion and O:N ratio are shown in Table 3. An increase in dietary CSM level did not significantly affect oxygen consumption ( $P > 0.05$ ). A reduction of oxygen consumption was observed while supplemental lysine is used, particularly in the CSM66+lys and CSM100+lys groups which were significantly lower than that in CSM0 treatment ( $P < 0.05$ ). Meanwhile oxygen consumption rate of crab fed CSM100+lys was significantly lower than crab fed CSM33 and CSM66 ( $P < 0.05$ ). Ammonia excretion of crab fed CSM0 diet was significantly higher than crab fed other diets ( $P < 0.05$ ). Crab fed CSM33+lys, CSM66+lys and CSM100+lys discharged less ammonia than those fed CSM33, CSM66 and CSM100 ( $P < 0.05$ ), but no significant differences were found between CSM33+lys, CSM66+lys and CSM100+lys, or between CSM33, CSM66 and CSM100 diets ( $P > 0.05$ ). The highest O:N ratio was observed in CSM33+lys treatment, which was significantly higher than that in CSM0, CSM33, CSM66, CSM100 and CSM100+lys treatments ( $P < 0.05$ ), but there were no significant differences between CSM0, CSM33, CSM66 and CSM100, between CSM33, CSM66, CSM100 and CSM100+lys, between CSM33+lys and CSM66+lys, or between CSM66+lys and CSM100+lys ( $P > 0.05$ ). The O:N ratio in the CSM66+lys diet was significantly higher than those in the CSM0, CSM33, CSM66 and CSM100 diets ( $P < 0.05$ ), and the O:N

ratio of crab fed CSM100+lys was significantly higher than that of crab fed CSM0 ( $P < 0.05$ ).

Specific activity of ASAT and ALAT data are presented in Figure 1. No significant difference was found in the specific activities of hepatopancreatic ALAT in the mitten crab fed with different diets ( $P > 0.05$ ). However, the specific activities of ASAT were significantly lower in crab fed CSM66+lys and CSM100+lys compared to crab fed CSM0, CSM33 and CSM100 ( $P < 0.05$ ), but there were no differences between CSM66, CSM33+lys, CSM66+lys and CSM100+lys, or between CSM0, CSM33, CSM66, CSM100 and CSM33+lys ( $P > 0.05$ ).

### Discussion

Many studies have evaluated the use of CSM as a source protein in the diet of various fish species. Robinson and Daniels (1987) evaluated glandless cottonseed meal (GLC) as partial and complete replacements for SBM in pond feeds for channel catfish. The results indicated final weight and feed conversion of fish fed the GLC feed were equal to those fed the control feed, even though the GLC feed appeared to be deficient in available lysine. Robinson (1991) reported that up to 100% of SBM could be replaced with CSM if supplemental lysine in feeds for channel catfish. Lee *et al.* (2006) found similar results that the growth performance of rainbow trout was not significantly affected during a 35-month trial when fish meal was completely replaced by CSM with lysine supplementation. Despite the successful use of CSM in fish diet formulation, but CSM is not commonly used in crustacean diets. Lim (1996) found that the performance of *Litopenaeus vannamei* was adversely affected when diets containing more than 26.5% CSM. In the present study, CSM could completely replace SBM without negative effects on growth performances and survival of juvenile Chinese mitten crab. It could be in part due to the tolerance to gossypol (Li and Robinson, 2006), amino acid composition of CSM, or higher digestibilities on amino acids of CSM than on SBM (Zhang *et al.*, 2007). The similar growth performance in high CSM inclusion to the control may be in part due to its high arginine content, an essential amino acid for aquatic animals. And Zhang *et al.* (2007) reported that the mitten crab *E. sinensis* could digest SBM protein better than CSM protein, but showed lower digestibilities on total amino acids, arginine, and methionine in SBM than in CSM. In contrast, *E. sinensis* showed similar digestibility on lysine in CSM or SBM diets. Therefore, using cottonseed meal as a partial protein source in crab feed can bypass adding crystalline arginine, and reduce the selective catabolism of amino acids in protein synthesis.

Aquatic animal ammonia excretion rate reflects the deamination of amino acids, and it is the best indicators to evaluate the effect of diet quality on the protein metabolism of aquatic animal (Thu, 2010).

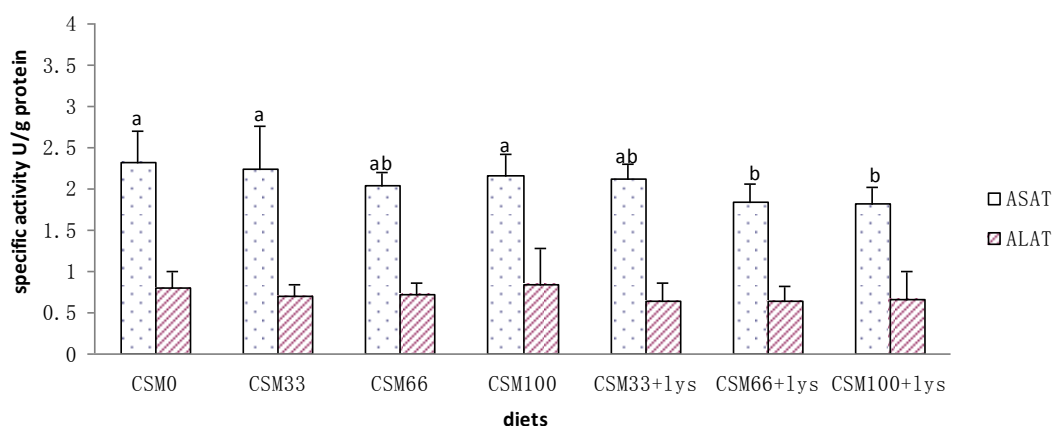
**Table 2.** Growth performance of juvenile Chinese mitten crab fed on experimental diets

Item	Experimental diets						
	CSM0	CSM33	CSM66	CSM100	CSM33+lys	CSM66+lys	CSM100+lys
IBM <sup>a</sup>	1.62±0.061	1.63±0.078	1.62±0.080	1.62±0.089	1.63±0.095	1.61±0.063	1.62±0.056
FBM <sup>b</sup>	3.54±0.074	3.59±0.41	3.27±0.12	3.43±0.18	3.57±0.36	3.39±0.16	3.40±0.16
WG <sup>c</sup>	118.77±4.55	120.25±25.20	101.60±7.39	111.60±11.17	118.90±21.89	110.43±10.04	109.88±10.16
SGR <sup>d</sup>	1.86±0.049	1.87±0.27	1.67±0.089	1.78±0.12	1.86±0.25	1.77±0.12	1.76±0.12
Survival <sup>e</sup>	93.33±7.64	95.00±5.00	93.33±2.89	96.67±2.89	93.33±2.89	95.00±5.00	93.33±2.89

<sup>a</sup>IBM (g crab<sup>-1</sup>), initial mean body weight<sup>b</sup>FBM (g crab<sup>-1</sup>), initial mean body weight<sup>c</sup>WG (%), weight gain<sup>d</sup>SGR(%/day<sup>-1</sup>), specific growth rate<sup>e</sup>Survival (%) = 100 × (final crab number)/(initial crab number)**Table 3.** Oxygen consumption, ammonia excretion and O:N ratio of juvenile Chinese mitten crab fed different diets

Item	Experimental diets						
	CSM0	CSM33	CSM66	CSM100	CSM33+lys	CSM66+lys	CSM100+lys
Oxygen consumption mg O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup>	0.10± 0.012 <sup>a</sup>	0.099± 0.0041 <sup>ab</sup>	0.095± 0.011 <sup>ab</sup>	0.088± 0.0073 <sup>abc</sup>	0.092± 0.0067 <sup>abc</sup>	0.086± 0.0075 <sup>bc</sup>	0.079± 0.004 <sup>c</sup>
Ammonia excretion µg NH <sub>4</sub> -N g <sup>-1</sup> h <sup>-1</sup>	2.91± 0.35 <sup>a</sup>	2.12± 0.33 <sup>b</sup>	2.14± 0.42 <sup>b</sup>	1.98± 0.50 <sup>b</sup>	1.02± 0.27 <sup>c</sup>	1.22± 0.38 <sup>c</sup>	1.20± 0.19 <sup>c</sup>
O:N ratio	39.50± 1.61 <sup>d</sup>	52.66± 0.83 <sup>cd</sup>	50.50± 3.36 <sup>cd</sup>	50.21± 4.45 <sup>cd</sup>	104.42± 25.98 <sup>a</sup>	83.84± 22.88 <sup>ab</sup>	74.3± 6.43 <sup>bc</sup>

Mean values ± SD in the same row sharing different letters are significantly different (P&lt;0.05).

**Figure 1.** Specific activity of hepatopancreatic aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) in *Eriocheir sinensis* fed on experimental diets (mean ± SD). Means within figure with the different letter are significantly different (P<0.05).

Engin and Carter (2005) reported that the lower daily NH<sub>3</sub>-N excretion rate of *Anguilla australis australis* was obtained on lupin meal (LM) diet when 100% SBM was replaced with lupin meal (LM), and meanwhile no significant differences in weight gain and specific growth rate were observed between two treatments. Viola and Lahav (1991) found that a reduction of nitrogen excretion per unit weight gain of common carp fed a diet containing a 25% crude protein, along with adding lysine, compared to fish fed with a 30% crude protein. Cheng *et al.* (2003) reported that rainbow trout fed with lysine-supplemented diets discharged less total ammonia

nitrogen (TAN) than fish fed with the basal diet. In the present study, crab fed CSM0 diet had higher ammonia excretion than crab fed other diets. Crab fed CSM33+lys, CSM66+lys and CSM100+lys discharged less ammonia than those fed CSM33, CSM66 and CSM100. Using cottonseed meal as a partial protein source in crab feed can bypass adding crystalline arginine, balance of amino acid profile of diet and reduce the selective catabolism of amino acids in protein synthesis. Likewise, adding lysine with an appropriate amount in diet can balance the amino acid profile in the diet.

Oxygen consumption rate is an index of

metabolic expenditure of animals to maintain their vital functions through aerobic metabolism (Valverde *et al.*, 2009). With oxygen consumption, it was possible to calculate apparent heat increment (AHI) which was a parameter that could be explained as a product of catabolism of protein, to quantify the energy losses through ingestion, digestion and assimilation of certain types of food (Suárez *et al.*, 2009). Du and Niu (2003) found that oxygen consumption of *Macrobrachium rosenbergii* fed the 75% SBM diet was significantly higher than fish meal-based diet. This was in agreement with result of Suárez *et al.* (2009), who reported that AHI values of *Litopenaeus vannamei* fed fishmeal based diet were lower than those fed other protein sources. Kaczanowski and Beamish (1996) observed that diets providing essential amino acids in similar proportions to those found in rainbow trout whole body protein could improve the efficient utilization of dietary amino acids and minimize energy liberated as AHI. In the present study, oxygen consumption rate of crab fed CSM66+lys and CSM100+lys was significantly lower than crab fed CSM0. Lysine supplementation reduced energy losses of crab used protein as metabolic substrates.

The O:N atomic ratio is a beneficial parameter to evaluate the nutrients used by animals as substrates of metabolic energy (Mayzaud and Conover, 1988). Perera *et al.* (2005) reported that lobsters *Panulirus argus* fed clam and chiton diets used protein for oxidation at 25% protein levels, according to the O:N ratio, while lobsters fed high-quality fish meal and squid diets used protein-lipid as energy substrates. *Litopenaeus vannamei* postlarvae had an O:N 60 signaling mixture of lipid and protein as metabolic substrate in fed animal protein, however the O:N ratio in the vegetable protein and carbohydrates treatment was lower than 20 (indicating an increase in protein use)(Jiménez-Yan *et al.*, 2006). In the present study, the O:N ratio in CSM0 was 39.50 demonstrating protein and some lipid as metabolic substrates. With the increasing level of CSM, the O:N ratios were 52.66 to 50.21 and it showed that *E. sinensis* used equal proportions of lipid and protein as energy substrates. These results demonstrated that the proportions of protein used as energy substances decreased when SBM was replaced by CSM. O:N values with supplemental lysine fed were higher than 60 (indicating lipid and carbohydrate as metabolic substrates). These increases might be related on a better dietary amino acid balance.

Aspartate aminotransferase (ALAT) and alanine aminotransferase (ASAT) are two enzymes, mostly active in liver and kidney, which are quantitatively important in transamination of amino acids (Hansen *et al.*, 2007). In the present study, although no clear effect of dietary treatment on specific activities of ALAT, SBM substitution by CSM resulted in an decrease of specific activities of ASAT, and lysine supplementation led to significantly lower specific

activities of ASAT in CSM66+lys and CSM100+lys treatments compared to CSM0, CSM33 and CSM100 treatments. The result could explain the decrease in ammonia excretion in these groups.

In conclusion, the growth performance, survival and ammonia excretion rate in Chinese mitten crab suggest that 100% of soybean meal can be replaced by cottonseed meal with lysine supplementation in the diets. And the addition of lysine decrease ammonia pollution discharge in the course of aquaculture. Future, the search for rational combination of low-cost plant protein sources and reduction of amino acids supplementation and ammonia excretion to replace SBM should be an increasing interest in the research of the mitten crab's nutrition.

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