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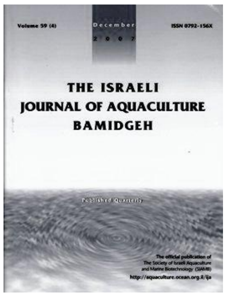


# Partial or Total Replacement of Soybean Meal by Cottonseed Meal in Practical Diets for Chinese Mitten Crab (*Eriocheir sinensis*): Effects on Growth, Feed Utilization, and Body Composition

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**Abstract**

This study investigated the replacement of soybean meal by cottonseed meal in diets for the Chinese mitten crab, *Eriocheir sinensis*. Seven isocaloric and isonitrogenous diets were tested with five replicates of each treatment. Cottonseed meal replaced 33%, 66%, or 100% of the soybean meal in three diets, while lysine was added to another three containing the same amounts of cottonseed meal. The control contained neither cottonseed meal nor lysine. Protein digestibility decreased with the increase of dietary cottonseed meal and was significantly lower in the 100% treatment than in the control. Dry matter in the body of crabs fed the 33%, 66%, 100%, and 33%+lysine diets was significantly lower than that of the control. Whole body protein decreased as the cottonseed meal content increased, and was significantly lower in crabs fed the 100% diet than in control crabs. Crabs fed the control diet contained higher lipid than those fed other diets, while crabs fed diets containing lysine had significantly lower lipid than crabs fed the 33% diet. Crabs fed the 100%+lysine diet had a significantly higher ash content than those fed the 33% or 100% diets. In conclusion, this study indicates that 66% of soybean meal can be replaced by cottonseed meal in crab diets and 100% of soybean meal can be replaced by cottonseed meal when supplemented by lysine.

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## Introduction

Although the Chinese mitten crab (*Eriocheir sinensis*) is an invader species in the USA and Europe (Rudnick et al., 2003; Herborg et al., 2005), it has long been a popular delicacy in China, Japan, and other Asian countries. Significant development has been made on the farming of this species and production in China increased from 8,000 tons in 1991 to 500,000 tons in 2004 (Yang and Zhang, 2005). Revenue from this species is $1.25 billion in China (Herborg et al., 2005) but profit is lowered because feed is a large proportion of the total production cost. The amount and type of proteins in aquaculture feeds account for the major cost of feed manufacture.

Soybean meal is the most common plant protein used to replace fishmeal in aquaculture feeds because of its high protein content, relatively well-balanced amino acid profile, reasonable price, and steady supply (El-Sayed, 1999). However, high demand for soybean meal has made it a relatively expensive protein source. Therefore, it is necessary to find alternatives to partially or completely replace soybeans and make plant protein more affordable (Robinson and Li, 2008).

Cottonseed meal is a plant protein source long used in high-protein diets for terrestrial and aquatic animals due to its high protein content and low price (Li and Robinson, 2006; Gatlin III et al., 2007). Cottonseed meal is the third largest oil-seed meal product in the world, after soybean and rapeseed (Lee et al., 2006) and a rich source of arginine, an essential amino acid for crabs. Cottonseed meal has been tested in rainbow trout (*Oncorhynchus mykiss*; Lee et al., 2006), channel catfish (*Ictalurus punctatus*; Robinson and Li, 1994, 2008), and hybrid tilapia (*Oreochromis niloticus* × *O. aureus*; Yue and Zhou, 2008). The amount of cottonseed meal that can be included in aquaculture diets depends primarily on the species, the level of gossypol, and available lysine (El-Saidy and Gaber, 2004). Gossypol in cottonseed meal binds to lysine and inactivates its biological availability (Robinson and Li, 2008). The mitten crab better digests crude soybean meal protein than crude cottonseed meal protein, but the digestibility of total amino acids, arginine, and methionine of soybean meal is lower than that of cottonseed meal. In contrast, *E. sinensis* showed similar digestibility of lysine from cottonseed and soybean diets (Zhang et al., 2007).

The present study evaluated the growth, feed utilization, and body composition of juvenile *E. sinensis* fed diets containing different levels of cottonseed meal as a substitute for soybean meal, with and without supplemental microcapsule lysine.

## Materials and Methods

*Diets.* Four isonitrogenous (~36% crude protein) and isocaloric (~12.3 kJ/g) diets were formulated with cottonseed meal replacing 0 (control), 33%, 66%, or 100% of the soybean meal (Table 1). An additional three diets containing 33%, 66%, or 100% cottonseed meal were supplemented with microcapsule lysine-HCl. Fish oil and soybean oil (v:v 1:1) were used as the lipid sources, while corn starch was used for carbohydrate. Cellulose was used as a filler and sodium carboxymethyl cellulose was used as a binder. Chromic oxide ($Cr_2O_3$) was used as an inert marker at a concentration of 0.5 g/100 g. Diets were prepared by mixing dry ingredients, then adding oil and water (v/w 40%) to form a soft dough. The ingredients were mixed to facilitate pelleting by a bibolt plodder (TS 12, East China Sea Fisheries Research Institute, Shanghai, China). Pellets (1.0 mm diameter) were extruded, air-dried to <10% moisture, and stored at -20°C. Diet stability was measured by placing five samples (~5 g) of each diet in petri dishes (100 mm diameter) immersed in aerated experimental tanks (20 cm deep water). After 2 h, the petri dishes with the wet diet were dried in an oven at 105°C to determine the amount of nutrient leached into the water.

*Animals and feeding.* Juvenile *E. sinensis* were obtained from Chongming Island Fisheries (Shanghai, China) and stocked in fifty 300-l polyvinyl tanks with 30 crabs each. Sixteen PVC tubes (50 mm diameter × 12 cm) and 10 tiles (15 × 20 cm) were placed in each tank to reduce animal aggression. Crabs were acclimatized for one week, then randomly restocked into thirty-five 300-l polyvinyl tanks at a density of 20 crabs (avg 1.62±0.089 g) per tank. The tanks were filled with 20 cm water and covered with a vinyl

Table 1. Formulation, proximate analysis, and water stability of diets for juvenile Chinese mitten crab in which cottonseed meal was used to replace soybean meal.

| | *Diet (% cottonseed meal)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Control* | *33%* | *66%* | *100%* | *33% +lysine* | *66% +lysine* | *100% +lysine* |
| Fishmeal | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Soybean meal | 60 | 40 | 20 | - | 40 | 20 | - |
| Cottonseed meal | - | 21 | 43 | 64 | 21 | 43 | 64 |
| Lysine-HCl (39%)[1] | - | - | - | - | 0.33 | 0.65 | 0.97 |
| Cellulose | 5.4 | 5.0 | 3.0 | 2.6 | 4.67 | 2.85 | 2.13 |
| Mineral mix[2] | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Vitamin mix[3] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Binder[4] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Corn starch | 2.5 | 1.5 | 1.5 | 0.5 | 1.5 | 1.0 | - |
| 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 |
| $Cr_2O_3$ | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| *Proximate composition (% dry matter)* | | | | | | | |
| 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 fiber | 13.42 | 14.60 | 14.53 | 14.53 | 15.04 | 13.63 | 14.74 |
| Gross energy (kJ/g)[5] | 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 |
| Water stability[6] | 65.96±1.94[c] | 70.14±2.80[b] | 72.25±2.08[b] | 78.91±2.02[a] | 70.41±3.09[b] | 73.48±0.79[b] | 80.11±0.78[a] |

Values in a row sharing the same superscript do not significantly differ ($p>0.05$).

[1] Microcapsule Lysine-HCl (Hinapharm Pharmaceutical Co. Ltd, Guangdong, China)

[2] Mineral mixture (%): $NaH_2PO_4\ 2H_2O$, 13.0; $KH_2PO_4$, 21.5; $Ca(H_2PO_4)_2\ H_2O$, 24.733; $CaCO_3$, 14.665; Ca-lactate, 16.5; $MgSO_4$, 4.88; $AlCl_3$, 0.945; $ZnSO_4\ 7H_2O$, 0.511; Fe-citrate, 0.061; $MnSO_4\ H_2O$, 0.108; KI, 0.058; $CuCl_2\ 2H_2O$, 0.0647; $CoCl_2\ 6H_2O$, 0.176; KCl, 2.8

[3] Vitamin mixture (Minshen Bio-tech Co. Ltd, Hangzhou, China) per 100 g mix: vitamin A, 420,000 IU; vitamin C, 6000 mg; vitamin E, 2000 mg; cholecalciferol, 120,000 IU; phytonadione, 1000 mg; thiamine, 1000 mg; riboflavin, 1000 mg; pyridoxine, 1600 mg; cobalamin, 2 mg; niacin, 5000 mg; folic acid, 400 mg; inositol, 6000 mg; biotin, 10 mg; calcium pantothenate, 3500 mg

[4] Sodium carboxymethyl cellulose (Shanghai Chemical, Shanghai, China)

[5] Gross energy calculation based on protein = 16.7 kJ/g, lipid = 37.6 kJ/g, NFE = 16.7 kJ/g (Ai et al., 2004)

[6] After immersion in freshwater for 2 h.

sheet to prevent the crabs from crawling out. Five replicate tanks were randomly assigned to each diet. Crabs were fed twice daily (08:00-09:00, 17:00-18:00) at a daily rate of 8-10% of their wet body weight for 10 weeks and weighed every three weeks to adjust the feed amount. Excess feed on tank bottoms was siphoned out 2 h after feeding, dried, and weighed to estimate feed consumption. Fecal samples were collected at 12:00-13:00 and 21:00-22:00 every day. Feces were collected for 7 weeks to obtain a sufficient amount for chemical analysis and stored at -20°C before analysis. Tanks were kept in ambient temperature (17-25°C) and photoperiod (11 h light:13 h dark), pH ranged 7.9-8.4, dissolved oxygen was <6.5 mg/l, and $NH_3$-N ranged 0.01-0.03 mg/l.

*Sample analysis and calculation.* Chemical analyses of formulated diets, feces, and crab samples were performed according to AOAC (1995). Feces and tissue samples were freeze-dried and homogenized before analysis. Crude protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method, crude lipid by ether extraction using Soxhlet, ash by combustion at 550°C, and crude fiber by the fritted glass crucible method after treatment with $H_2SO_4$ and NaOH. Nitrogen-free extract was estimated on a dry weight basis by subtracting the percentages of crude protein, lipid, fiber, and ash from 100%. $Cr_2O_3$ content was analyzed using a wet-acid digestion method (Furukawa and Tsukahara, 1966). Analyses were performed in triplicate. Growth performance was determined by weight gain and specific growth rate (SGR). Feed utilization was evaluated by feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV).

*Statistical analysis.* Results are expressed as means±standard deviation. All data were compared using one-way analysis of variance (ANOVA) and mean differences were compared using Duncan's multiple range test. Differences were considered significant when $p<0.05$. Statistical analyses were performed using SPSS11.5.

## Results

The dietary treatments had little significant effect on survival, growth, and feed utilization (Table 2). Protein and lipid contents in the mitten crab body dropped with the increase of cottonseed meal.

Table 2. Growth, feed utilization, survival, apparent digestibility coefficients, and body composition of juvenile Chinese mitten crab fed diets containing different levels of cottonseed meal and lysine.

| | *Diet (% cottonseed meal)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Control* | *33%* | *66%* | *100%* | *33%+lysine* | *66%+lysine* | *100%+lysine* |
| Initial body wt (g) | 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 |
| Final body wt (g) | 3.78±0.14 | 3.81±0.52 | 3.54±0.20 | 3.61±0.16 | 3.79±0.26 | 3.61±0.11 | 3.80±0.35 |
| Wt gain (%)[1] | 134.82±11.13 | 136.55±32.71 | 118.71±18.76 | 123.07±16.93 | 135.49±17.18 | 125.17±17.26 | 134.58±28.97 |
| SGR (%/day)[2] | 1.22±0.069 | 1.22±0.19 | 1.11±0.12 | 1.14±0.11 | 1.22±0.11 | 1.16±0.11 | 1.21±0.18 |
| FCR[3] | 1.85±0.28 | 2.03±0.51 | 2.24±0.71 | 1.97±0.51 | 2.09±0.41 | 2.11±0.52 | 2.13±0.14 |
| PER[4] | 0.98±0.0026 | 0.98±0.0029 | 0.98±0.0013 | 0.98±0.0015 | 0.98±0.0014 | 0.98±0.0012 | 0.98±0.00074 |
| PPV (%)[5] | 28.35±3.65 | 24.90±4.17 | 22.50±5.07 | 23.40±3.96 | 24.63±2.10 | 23.05±4.04 | 23.67±1.00 |
| Survival (%) | 90±8.67 | 93.33±7.64 | 91.67±2.89 | 95.00±0.00 | 90.00±0.00 | 93.33±5.77 | 90.00±0.00 |
| *Apparent digestibility coefficients (ADC; %)* | | | | | | | |
| Dry matter[6] | 79.57±1.25 | 79.41±1.86 | 78.68±1.60 | 77.00±4.11 | 78.86±1.77 | 78.52±1.10 | 78.21±2.40 |
| Protein[7] | 87.01±0.80[a] | 85.90±1.27[ab] | 85.10±1.12[ab] | 84.47±2.77[b] | 85.35±1.23[ab] | 84.98±0.76[ab] | 84.79±1.67[ab] |
| Lipid[7] | 82.34±1.08 | 80.44±1.77 | 80.82±1.44 | 81.98±3.22 | 80.36±1.64 | 80.25±1.00 | 81.66±2.02 |
| *Whole body composition (%)* | | | | | | | |
| Dry matter | 37.64±0.49[a] | 36.32±0.71[c] | 36.42±0.31[c] | 36.41±0.67[c] | 36.41±0.19[c] | 37.10±0.75[ab] | 37.22±0.32[ab] |
| Protein | 14.68±0.60[a] | 14.36±0.61[ab] | 13.78±0.18[ab] | 13.51±0.83[b] | 13.99±0.15[ab] | 13.95±0.39[ab] | 14.28±0.097[ab] |
| Lipid | 5.95±0.46[a] | 5.07±0.45[b] | 4.79±0.072[bc] | 4.75±0.10[bc] | 4.38±0.26[c] | 4.51±0.21[c] | 4.48±0.089[c] |
| Ash | 13.80±0.20[ab] | 13.48±0.29[b] | 13.76±0.13[ab] | 13.44±0.077[b] | 13.69±0.42[ab] | 13.68±0.26[ab] | 14.06±0.050[a] |

Values in a row sharing the same superscript do not significantly differ ($p>0.05$).
[1] Weight gain = 100(final body wt - initial body wt/initial body wt)
[2] Specific growth rate = 100($\ln_{final\ wt} - \ln_{initial\ wt}$)/days of experiment
[3] Feed conversion ratio = dry feed consumed/wet wt gain
[4] Protein efficiency ratio = wet wt gain/protein intake
[5] Protein productive value = 100(protein gain/protein intake)
[6] ADC of dry matter = 100 - 100(%$Cr_2O_3$ in diet/$Cr_2O_3$ in feces)
[7] ADC of protein and lipid = 100 - 100(%$Cr_2O_3$ in diet/$Cr_2O_3$ in feces)(%nutrient in feces/%nutrient in diet)

## Discussion

Glandless cottonseed meal is a suitable substitute for soybean meal in channel catfish diets, even if the feed is deficient in lysine (Robinson and Daniels, 1987). Soybean meal can be replaced by cottonseed meal up to 30% without lysine supplementation and up to 100% with lysine supplementation in channel catfish diets (Robinson, 1991). Similarly, growth performance of rainbow trout was not significantly affected during a 35-month trial when fishmeal was completely replaced by cottonseed meal supplemented with lysine (Lee et al., 2006). However, despite its successful use in fish diets, replacement of soybean meal by cottonseed meal in crustacean diets is uncommon. *Litopenaeus vannamei* are adversely affected when diets contain more than 26.5% cottonseed meal (Lim, 1996). In the present study, cottonseed meal completely replaced soybean meal without negative effects on growth performance, feed utilization, or survival, suggesting that Chinese mitten crabs have high tolerance to gossypol (Li and Robinson, 2006). Survival of mitten crabs improved when trace elements such as copper and zinc were added to the diet (Sun et al., 2011). A benefit of cottonseed meal as a plant protein is that its content of arginine, an essential amino acid for mitten crabs, is higher than that of fishmeal and soybean meal. Using an appropriate amount of cottonseed meal in crab feeds can prevent the need to add crystalline arginine. Further, cottonseed meal produces a better dietary amino acid balance, which can probably reduce the selective catabolism of amino acids and consequently increase protein synthesis.

The ADC of protein decreased as the dietary cottonseed meal level increased, and was significantly lower in the 100% diet than in the control. A similar negative correlation between protein ADC and dietary cottonseed meal was found in Nile tilapia (El-Saidy and Gaber, 2004), hybrid tilapia (Yue and Zhou, 2008), and rainbow trout (Luo et al., 2006). Protein digestibility is low not only in mitten crab, but also in fish. The ADC of crude protein for soybean meal is 77.94%, significantly higher than 71.63% for cottonseed meal (Zhang et al., 2007). Despite the lower protein digestibility of crabs fed the cottonseed meal diets, the growth of the crabs fed the 100% diet did not differ from that of the crabs fed the control diet, possibly because the digestibility of total amino acids, arginine, and methionine of cottonseed meal is higher than that of soybean meal in *E. sinensis* (Zhang et al., 2007). Our study suggests that protein ADC of cottonseed meal-based diets can be improved by lysine supplementation, in agreement with studies on protein digestibility of rainbow trout fed cottonseed meal diets with lysine supplementation (Cheng et al., 2003; Luo et al., 2006). Lysine supplementation in diets can balance the composition of dietary amino acids and increase protease activity in the stomach and gut (Zhang et al., 2009), resulting in improved digestibility and absorption of dietary protein.

Whole body ash content was not affected by the level of dietary cottonseed meal, but dry matter, protein, and lipid contents decreased as the dietary cottonseed meal increased. Pacific white shrimp (*L. vannamei*) fed 0, 13.3%, or 26.5% cottonseed meal had similar weight gains, feed consumptions, and survival, but when the cottonseed meal was increased to 39.8%, the shrimp had lower weight gains, feed intakes, and survival (Lim, 1996). Similarly, when the replacement of soybean meal by cottonseed meal reached 55% in feeds for channel catfish (*I. punctatus*), weight gain, feed intake, and feed efficiency ratio decreased (Barros et al., 2002). In our study, the reduction of whole body protein at high replacement levels could be due to low digestion efficiency of protein, even though the whole body protein contents of crabs fed the 33% or 66% diets were similar to the control. Cottonseed meal is rich in arginine, which may facilitate protein synthesis in the same way that dietary arginine supplementation enhanced growth in red-sea bream larvae (Lopezalvarado and Kanazawa, 1994) and rainbow trout (Rodehutscord et al., 1995).

The addition of lysine to the crab diets increased the dry matter contents of the whole body, particularly up to the 66% cottonseed meal level, as reported for grass carp *Ctenopharyngodon idella* (Wang et al., 2005), and Jian carp (*Cyprinus carpio* var. Jian; Zhou et al., 2008). The whole body moisture content of *C. idella* decreased with the increase of lysine up to 2.18% (Wang et al., 2005), likewise, whole-body moisture was lower in Jian carp fed diets containing 41-69 g lysine/kg protein than in carp fed the control containing only 22 g lysine/kg protein (Zhou et al., 2008).

Changes in the whole body protein and lipid of crabs fed the diets with supplemented lysine agree with results in yellowtail *Seriola quinqueradiata* (Ruchimat et al., 1997) and Japanese seabass *Lateolabrax japonicus* (Mai et al., 2006). Body protein of *S. quinqueradiata* increased as the dietary lysine level increased to 1.85% of the dry diet, while fat content showed an opposite trend (Ruchimat et al., 1997). Similarly, whole body protein of *L. japonicus* positively correlated with the dietary lysine level, while lipid negatively correlated (Mai et al., 2006), demonstrating that a better dietary amino acid balance may reduce selective catabolism of amino acids, consequently increasing protein synthesis and decreasing lipid accumulation in tissues (Benevenga et al., 1993). Dietary lysine supplementation may improve lipid metabolism in crab because lysine is essential for the synthesis of carnitine that is involved in the transport of fatty acids across mitochondrial membranes, and facilitated oxidation of the fatty acids to produce energy and reduce the deposition of body fat (Dias et al., 2001). In this study, lysine supplement increased the ash content of the whole body, similar to results in Jian carp (Zhou et al., 2008).

In conclusion, 66% of soybean meal can be replaced by cottonseed meal in diets for Chinese mitten crab without compromising growth, feed utilization, or body composition. Further, soybean meal can be totally replaced by cottonseed meal with lysine supplementation. However, the addition of lysine increases feed costs that may reduce the economic advantages of using cottonseed meal. Research into a rational combination of low-cost plant protein sources and reduction of amino acid supplementation that can replace soybean meal should be of increasing interest in the development of mitten crab nutrition.

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