クルマエビ(Penaeus japonicus)の成長と消化率に及ぼすエクストルーダー処理脱脂大豆の効果

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The Effects of Extruded Soybean Meal on Growth and Digestibility of Kuruma Shrimp, *Penaeus japonicus* Juveniles

Sanshiroh Saitoh *,1, Shunsuke Koshio *,2,4, Hiroshi Harada *,1, Kazunori Watanabe *,3, Takeshi Yoshida *,3, Shin-ichi Teshima *,2, and Manabu Ishikawa *

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**Abstract:** The growth trial of kuruma shrimp, *Penaeus japonicus*, was conducted to determine the nutritional value of twin screw-extruded soybean meals (EX-SBM) and to investigate the optimum replacement level of EX-SBM with fishmeal. Five isoenergetic and isonitrogenous test diets were formulated to contain EX-SBM was increased from 0 to 32% while fishmeal was reduced from 39 to 18% correspondingly in the trials. Two feeding trials with 0.5 g (trial 1) and 5g (trial 2) of initial body weight were conducted, in which animals were fed test diets for 30 days at the level of 8-10% body weight and 4-5% body weight, respectively.

In trial 1 and 2, kuruma shrimp grew well with low mortality and animals fed the diets containing 8 to 32% EX-SBM showed very similar growth performance to that obtained in the fishmeal group. The present study demonstrated that the growth of *Penaeus japonicus* was not retarded at the level of 32% EX-SBM in the diet.

This study demonstrated that EX-SBM, which contains very low antigen, could be a potential candidate for fishmeal replacement in crustacean feeds.

**Key words:** Antigenicity; Soybean meal; Kuruma shrimp; Twin-screw extrusion

The demand for plant proteins, especially soybean products in aquaculture feeds, has been growing as a replacement for fishmeal, which is becoming more and more expensive. It has been demonstrated in previous studies that diets with soybean meal (SBM) induced low growth performance, which caused unsatisfactory amino acid profiles, and included anti-nutritional substances such as trypsin inhibitors, lectins and antigens, for aquatic animals1,2). In cases of young land animals, the utilization of SBM is limited due, in part, to the antigenicity, which may cause severe diarrhea and growth stunting3,4). Recently, it was demonstrated that the soybean antigen contents could be quantitatively analyzed and be reduced by twin-screw extrusion5), and diarrhea and growth retardation were prevented by feeding the diets containing lower contents of soybean antigens6). However, there are very limited studies using SBM with low antigen levels on aquatic animals. The aim of this study is to evaluate the nutritional values and determine the optimal dietary level of SBM containing a very low antigenicity (6.7 U/10 mg) for kuruma shrimp, *P. japonicus*, which is one of the most important aquaculture crustaceans in Japan.

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Materials and Methods

Production of Twin Screw-Extruded Soybean Meals (EX-SBM)

Soybean meal (SBM) was supplied from Honen Corporation (Tokyo, Japan). Twin screw-extruded soybean meals (EX-SBM), which were cooked in a KEI-45 co-rotating twin-screw extruder (Kowa Kogyo Co., Osaka, Japan), were prepared under the following conditions. Ten ports die with 2.0 mm each and screws combined 6 blocks of kneading discs in the heads, were used for the production. Screw speed, amounts of water and soybean meals supplied, and the barrel temperature were 280 rpm, 12 l/hr and 51.4 kg/h, and 134 º, respectively. The barrel temperature was controlled by electric heating with a band heater and water-cooling. Extruded materials were dried at 80 º for 15 min in the oven and ground to pass through a 100-mesh screen. Amounts of antigen and trypsin inhibitor were measured according to Ohishi et al.5). The analytical comparisons between EX-SBM and SBM are shown in Table 1.

Experimental Diets

According to the report of Lim and Dominy 7), it was thought that more than 28% of SBM, as a substitute for fishmeal, would lead to a low growth rate for shrimps. To evaluate optimum level of EX-SBM for alternative protein source with fishmeal, five isoenergetic and isonitrogenous test diets (Table 2) were formulated to contain 0 (control) to 32% of EX-SBM concomitant with decrease of fishmeal from 39 to 18% correspondingly in the trials. Other protein sources were squid powder and krill meal. Test diets were produced according to the method of Koshio et al.8), and then pellet type diets

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<th>Measured parameters</th>
<th>EX-SBM (%)</th>
<th>C-SBM *1 (%)</th>
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<tbody>
<tr>
<td>Moisture (%)</td>
<td>5.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Crude protein (%) *2</td>
<td>49.2</td>
<td>48.2</td>
</tr>
<tr>
<td>Crude fat (%) *2</td>
<td>1.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Ash (%) *2</td>
<td>6.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Trypsin inhibitor (U/mg)</td>
<td>1.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Antigenicity (U/10mg)</td>
<td>6.7</td>
<td>10000</td>
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*1 Honen Corporation.
*2 Dry weight basis.
were produced through a meat grinder (Hiraga Seisakusho, Japan), after mixing dry ingredients and water (2:1, w/v), with a 1.2 mm die in trial 1 and 3 mm die in trial 2, respectively. Formulated diets were oven-dried to approximately 5% moisture and stored at -20°C until used.

Feeding Trial

Juvenile shrimps, which were obtained from Mitsui Norin Farm, were placed in a round holding tank (0.5 t) and fed a commercial feed (Higashimaru Co. Ltd., Kagoshima, Japan) under ambient temperature (21-28°C) and salinity (32 °) until the shrimps were used in the trials. Two trials were conducted using small (trial 1) and medium size (trial 2) juvenile shrimps.

In trial 1, a total of 300 juveniles (initial size about 0.5 g) were randomly selected from the holding tank. Twenty shrimps per tank were transferred and communally housed in a 54 liter rectangular PVC tank (60 x 30 x 30 cm) with sand bottoms (5 cm thickness) with triplicate tanks per treatment (total 60 shrimps per treatment). Water system was the flow through, and filtered seawater was continuously supplied at a flow rate of 3 l/h together with a net under the sand. The water volume in each tank was kept at 45 liters. The growth trial was conducted at 26-28°C under a predominantly dark condition, with occasional lighting when mortality was checked, or when feeding or periodic weight measurements were carried out. Shrimps were fed once a day at 17:00, on a ration equal to 8-10% of body weight per day for 30 days. The ration was adjusted after every 10 days weighing. The tanks, sand and nets were cleaned after shrimp were removed for each weight measurement. Uneaten diets were removed from the tank every morning and dried at 100°C for 24 h for determination of virtual food intake. All animals were blotted on paper towels for 1 min to remove excess water and then weighed using an electro-balance (model AEL 200, Shimadzu Co. Ltd., Kyoto, Japan).

After collecting the growth data, the same test diets were continued as for those in the growth experiment except for the inclusion of Cr₂O₃ (1% in the diet) for another month for the digestibility determinations. Thirteen shrimps from each dietary treatment were randomly chosen and placed in the feeding tank with duplicate. The rearing was the same manner as in the growth trial. Animals were allowed to feed the test diets at 5% of body weight to produce enough feces for the analysis. After feeding, the shrimps were transferred to the feces-collection tank, and feces were collected by pipetting 6 hours after the transfer. Feces were freeze-dried immediately after collecting. Chromium contents were analyzed according to Furukawa and Tsukahara9).

In the trial 2, a total of 395 juveniles (initial size about 4.7 g) were randomly selected from the holding tank. Thirteen shrimps per tank with triplicate tanks per treatment (total 39 animals per treatment) were communally housed in the rearing tanks. The rearing methods were similar to those in trial 1 except for the following conditions: water temperature, 21-22°C; ration size, 4-5% of body weight per day. After the termination of growth trial, dry matter and protein digestibility were also measured by the similar manner to trial 1.

Measured Parameters for Nutritional Evaluation of EX-SBM

The following parameters were calculated for the nutritional evaluation of EX-SBM:

digestibility of dry matter (％) =
\[
(1 - \%Cr₂O₃ \text{ in diet}/ \%Cr₂O₃ \text{ in feces}) \times 100
\]
digestibility of protein (％) =
\[
(1 - \%\text{protein in feces}/ \%\text{Cr₂O₃ in feces}) \times \%\text{protein in diet}/ \%\text{Cr₂O₃ in diet} \times 100
\]

Chemical Analyses

Moisture, protein, lipid, and ash contents of the EX-SBM, commercial SBM and prepared test diets were determined by the methods of the Association of Official Analytical Chemists10). The gross energy values of test diets were measured by bomb calorimeter (Model 100, Ogawa Sampling Ltd.), and amino acid analysis was
conducted by the method described by Teshima et al.1). Analytical results of test diets were shown in Tables 2 and 3.

Statistical Analysis

Statistical significance of differences among measured parameters was computed using analysis of variance (ANOVA) (Super Anova, Abacus Concepts, Inc.). Tukey-Kramer (Super Anova, Abacus Concepts, Inc.) was applied to determine significant differences between individual treatments when significance (P < 0.05) of factors was detected by ANOVA.

Results

Growth Performances of Kuruma Shrimp Fed EX-SBM Containing Diets

All test diets used in this study were identical besides the content of histidine and methionine where it decreased with added EX-SBM (Table 3). Tryptophan would not be measured exactly because of inhibition by carbohydrate in the diets. The results of trials 1 and 2 were summarized in Table 4. Survival rates were more than 80% among all diet groups in both experiments, and not affected by the level of dietary EX-SBM. In trial 1, all shrimps that were fed the control diet or test diets grew similarly and well (Fig. 1), in which weight gain reached about 350% (Table 4). In trial 2, shrimps also grew similarly in all diets (Table 4). In both growth experiments, a statistical significance was not detected on the final weight, body weight gain, daily feed intake, feed conversion efficiency, and protein efficiency ratio (Table 4).

Digestibility

Dry matter and protein digestibility in trials 1 and 2 were presented in Table 5. In the trial 1, higher value of dry matter digestibility was obtained in the fishmeal based diet, and it decreased with the addition of dietary EX-SBM.
but statistical significance was not detected in all groups. On the other hand, protein digestibility of the diet containing 8% EX-SBM was significantly higher than that of the control diet and the 24% EX-SBM diet. In trial 2, there was no significant difference on dry matter digestibility. Protein digestibility of diet containing 8% EX-SBM was significantly higher than that of other EX-SBM diets, although no significant difference from that of the control diet was obtained.

**Discussion**

This study demonstrated that EX-SBM could be a potential candidate for fishmeal replacement for crustacean feeds. In trial 1, the growth rates of all groups were higher than those in other kuruma shrimp trials using similar size of animal\(^{12,13}\), in which the major dietary protein source was crab protein concentrate. It has been reported that crab protein concentrate is a good source of protein for crustaceans\(^{12,13}\). When considering these results, it appears that test diet in the present experiment seem to provide good growth performance in shrimps.

Lim and Dominy\(^7\) reported that a 28% dietary level of SBM, as 40% replacement of the marine animal protein mixtures which were fishmeal, shrimp meal and squid meal, appears to be optimal based on the growth in juvenile *Penaeus vannamei*. In the present study, it is likely that up to 32% of EX-SBM, 67% replacement of the fishmeal, would be the allowance levels for growth in kuruma shrimp juveniles without any retardant. Feed intake and digestibility were not negatively affected by feeding the diet containing EX-SBM, which would, in part, contribute to the similar growth in all diet groups. Furthermore, by increasing dietary EX-SBM with lowered levels of dietary amino acids such as histidine and methionine would still meet the requirement of kuruma shrimp.

The treatment temperature of Ex-SBM is one of the keys that should be considered. As Shimeno *et al.*\(^{14,15}\) pointed out, excessive heat to SBM would cause a destruction of amino acids.
acids, such as arginine and lysine, resulting in poor growth of fishes. In the present study, the contents of arginine and lysine in EX-SBM were similar to those in non-treated SBM (as estimated from Table 3). Therefore, the extrusion treatment in this study is considered to be proper for the treatment of SBM.

In aquaculture feed, there are a few reports that refer to soy antigenicity. In the case of yellowtail, low growth rates happened when heated or extruded full-fat soybean diets were fed to yellowtail, compared to fishmeal\(^{15}\). The antigen levels of the heated and extruded full-fat soybeans in that study were 319 U/mg and 308 U/mg, respectively. In this study, the similar growth between control and experimental groups was observed. The result would be due to a low level of antigenicity (6.7 U/10 mg) and/or trypsin inhibitor (1.1 U/mg) in EX-SBM (Table 1).

On the other hand, Refstie reported in the study of 100 g size Atlantic salmon which were fed a low antigenicity SBM diet grew similarly to those fed a fishmeal based diet\(^{16}\). Furthermore, the salmon grew faster than that fed the diet containing toasted and extracted SBM. Meanwhile, Rumsey considered that antigenic soya protein affects non-specific defense mechanisms, growth performance, and protein utilization in rainbow trout\(^{15}\). When considering these articles, it was suggested that the antigenic soybean protein affected fish growth when it was not adequately removed. Therefore, the degradation of soybean protein in EX-SBM seems to be sufficient for shrimp growth.

Although there are several reports on the use of twin-screw extruders on the treatment of SBM in fish nutrition studies, the operation conditions were varied in each report. Therefore, the results are not conclusive at the present. Raw soybeans or commercial SBM contain the antigen approximately 20,000 U/10 mg and 10,000 U/10 mg, respectively. It could, however, decrease to approximately one tenth of the amount by heat treatment\(^{5}\). It has been demonstrated that the solvent treated soybean protein concentrates, with antigen level would be expected at around 26 U/10 mg\(^{5}\), produced good growth of kuruma shrimp larvae and freshwater shrimp juveniles\(^{18,19}\). In the present study, the antigen level of SBM was decreased to 6.7 U/10 mg, which is lower than the solvent treated soybean protein concentrate. Therefore, it is suggested that the antigen level of the EX-SBM was low enough to prevent the retardation of growth in kuruma shrimp.

In summary, EX-SBM with low antigen (6.7 U/10 mg) can be a satisfactory alternative protein source for kuruma shrimp. It will be expected that EX-SBM can be effectively utilized by other fishes as well, though additional research on other fish species would be needed.

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Effects of Low Antigenicity Soybean Meal


クルマエビ（Penaeus japonicus）の成長と消化率に及ぼすエクストラダーレ処理脱脂大豆の効果

齋藤三四郎・越塩俊介・原田 宏・渡部一憲
吉田岳史・手島新一・石川 学

2軸エクストラダーレ処理脱脂大豆（EX-SBM）の飼料価値を評価し、魚粉の代替率を検討する目的で、クルマエビ、Penaeus japonicusの成長試験を行った。EX-SBMはニーディングディスクを用いた軸を使用して処理を施し、大豆の抗原性を6.7U/10 mgまで低減した。飼料中の魚粉の含有量を39%18%に、EX-SBMの含有量を0-32%に変化させた5つの飼料を調製し、0.5g, 5gの各サイズのクルマエビに対して、それぞれ体重の8-10%, 4-5%の飼を給餌する2回の飼育試験を行った。

試験1, 試験2ともに成長が良く、飼育率も低く、EX-SBMを含有する全ての飼料区において対照魚粉区と同様の成長が見られた。以上の結果から、抗原性が低減した脱脂大豆は32%まで魚粉と置き換えても成長の阻害が起こらず、甲殻類の飼として魚粉の代替となり得ることが明らかとなった。