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# Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp *Litopenaeus schmitti* (Pérez-Farfante & Kensley 1997)

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## Abstract

The feasibility of substituting soybean meal for fishmeal diets for juvenile white shrimp *Litopenaeus schmitti* (0.35 ± 0.01 g) was evaluated, and an adequate substitution level was determined. Five diets were evaluated using 46%, 59%, 75%, 88% and 100% substitution levels. Pellet water stability was significantly affected by dietary soybean content ($P < 0.05$). Increased soybean content produced lower pellet stability, ranging from a dry matter loss of 14–22% after a 2-h immersion, and 20–33% after an 8-h immersion. After 52 days, significant differences ($P < 0.05$) were found in shrimp weight, feed conversion ratio and protein efficiency ratio. The values were 0.64–1.06 g, 2.8–7.9 and 0.45–1.21, respectively, for the three measurements. Overall, better results were obtained with diets where soybean meal was substituted for fishmeal up to 75%. The 100% soybean meal diet resulted in poor growth performance of shrimp. Survival rates were acceptable for all treatments (90% or higher) and no significant differences were found in survival between treatments. Regression analysis using the broken-line methodology indicated that 76.5 ± 2% is an optimum soybean substitution level in diets that contained fishmeal and soybean as the major protein sources for grow-out of juvenile white shrimp.

**Keywords:** shrimp, *Litopenaeus schmitti*, nutrition, fishmeal, soybean meal

## Introduction

Fishmeal is a preferred ingredient in balanced rations for shrimp because of its high protein content, essential amino acid composition (particularly lysine and methionine), ω3 fatty acids, mineral content and acceptable palatability and digestibility (Zaldivar 2002). Estimated demand for fishmeal will increase from 372 000 to 485 000 tonnes during the present decade, solely for manufacturing feedstuff for shrimp (Barlow 2000). Substitutes for fishmeal, which provide adequate nutrition and are economically feasible, need to be found (Smith, Allan, Williams & Barlow 2000).

Some researchers have reported that the nutritional value of soybean (*Glicine maxima*) meal is lower compared with fishmeal for penaeid shrimp (Tacon 1989; Zaldivar 2002). However, it is relatively inexpensive and has acceptable protein and amino acid content, making it a potential substitute in practical diets. Nutritional imbalances from using soybean meal in diets can be compensated by including complementary ingredients (Berger 2001; Forster, Dominy & Tacon 2002; Swick 2002). The nutritional response of aquaculture organisms to inclusion of soybean meal in diets is highly variable and depends, among other things, on the species, size, level of inclusion, ingredient composition and protein level (Lim & Dominy 1990; Pascual, Cruz- Suárez & Sumalangcay Jr 1990; Berger 2001).

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**Table 1** Composition and proximate analysis (% dry matter) of diets with soybean meal substituted for fishmeal fed to juvenile white shrimp *Litopenaeus schmitti*

| | Experimental diets (%) | | | | |
|---|---|---|---|---|---|
| Ingredients | S46 | S59 | S75 | S88 | S100 |
| Fishmeal* | 29 | 22 | 13 | 6 | 0 |
| Soybean meal† | 25 | 32 | 41 | 48 | 54 |
| Wheat meal‡ | 36 | 36 | 36 | 36 | 36 |
| Sunflower oil§ | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Fish oil¶ | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Vitamins and minerals‖ | 2 | 2 | 2 | 2 | 2 |
| Calcium carbonate** | 3 | 3 | 3 | 3 | 3 |
| Dicalcic phosphate** | 2 | 2 | 2 | 2 | 2 |
| Crude protein | 32.6 ± 0.08 | 31.8 ± 0.11 | 29.9 ± 0.09 | 28.6 ± 0.08 | 28.0 ± 0.06 |
| Total lipid | 6.0 ± 0.02 | 5.6 ± 0.06 | 5.4 ± 0.04 | 5.3 ± 0.02 | 5.1 ± 0.02 |
| NFE†† | 35.4 ± 3.6 | 36.9 ± 3.4 | 39.0 ± 3.5 | 40.5 ± 3.4 | 41.9 ± 3.1 |
| Ash | 17.5 ± 1.8 | 17.1 ± 1.7 | 16.6 ± 1.8 | 16.2 ± 1.7 | 15.9 ± 1.6 |
| Crude fibre | 2.9 ± 0.09 | 3.2 ± 0.06 | 3.6 ± 0.06 | 4.1 ± 0.08 | 4.3 ± 0.06 |

**Engraulis* sp. (Corpesca, SA, Chile).
†Defatted soybean meal (Cherry, Santiago de Cuba, Cuba).
‡Rice Co., Greenbrae, CA, USA.
§Empresa de aceites y grasas, Ciudad Habana, Cuba.
¶Corpesca.
‖Commercial formula (kg premix tonne$^{-1}$ diet). Unión de Empresas de Piensos MINAGRI, Cuba. Vitamins: retinol, 12 500 000 IU; thiamine, 10 000 mg; riboflavin, 20 000 mg; pyridoxine, 10 00 mg; cyanocobalamine, 40 mg; ascorbic acid (Stay C), 500 000 mg; cholecalciferol, 2 400 000 IU; α tocopherol, 100 000 mg; pantothenic acid, 40 000 mg; choline chloride, 1 600 000 mg; folic acid, 2000 mg; nicotinic acid, 140 000 mg; biotin, 1000 mg; inositol, 300 000 mg; paraminobenzoic acid, 35 000 mg. Minerals (mg): cobalt, 200; copper, 2000; iron, 20 000; iodine, 1500; manganese, 40 000; zinc, 20 000; selenium, 100.
**Cargill, Minneapolis, MN, USA.
††NFE, Nitrogen-free extract.

Soybean meal has been used as a substitute for fishmeal in diets of several crustaceans, including freshwater shrimp *Macrobrachium rosenbergii* (Tidwell, Webster, Yancey & D'Abramo 1993; Du & Niu 2003), American lobster *Homarus americanus* (Floreto, Bayer & Brawn 2000), Australian redclaw crayfish *Cherax quadricarinatus* (García-Ulloa, López, Rodríguez & Villarreal 2003) and penaeid shrimp, including *Penaeus monodon* (Sudaryono, Hoxey, Kailis & Evans 1995) and *Litopenaues vannamei* (Davis & Arnold 2000; Mendoza-Alfaro, De Dios, Vázquez, Cruz-Suárez, Ricque-Marie, Aguilera & Montemayor 2001). Soybean meal inclusion in the diet did not affect the growth of *Litopenaeus schmitti* (Lawrence, Castille, Sturmery & Akiyama 1986). However, the effect of varying soybean meal levels in the diet was not determined.

In this study, we evaluated the grow-out response of juvenile *L. schmitti* to five substitution levels of soybean meal for fishmeal in the diet. We also estimated an adequate substitution level.

## Materials and methods

### Preparation of diets

Five diets, each with different levels of soybean meal substitution, were prepared. The base diet was a commercial formulation where 46% (designated as S46) substitution (29% fishmeal and 25% soybean meal) was used for grow-out of *L. schmitti* (S46). For the other diets, the substitutions were 59%, 75%, 88% and 100% (designated as S59, S75, S88 and S100 respectively; Table 1). Crude protein (28.0–32.6%) and lipids (5.1–6.0%) were within the range considered to be optimal for *L. schmitti* (Galindo 2000). The chemical composition of the main protein sources is presented in Table 2.

Dietary ingredients were ground and passed through a 0.25-mm mesh sieve and homogenized for 3 min in a domestic blender (Hobart M-600, Hobart Corp., Troy, OH, USA). Fish oil and sunflower oil were gradually added, and warm water (approximately 50% of the total weight) was added during

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**Table 2** Dry weight composition (%) of the main protein sources used in the experimental diets for juvenile white shrimp *Litopenaeus schmitti**

| Ingredient | Crude protein | Ether extract | Crude fibre | Ash | NFE† |
|---|---|---|---|---|---|
| Fishmeal | 62.0 | 6.5 | 1.1 | 21.8 | 8.6 |
| Soybean meal | 44.2 | 2.8 | 5.9 | 16.2 | 30.9 |

*Values are means of three determinations.
†Nitrogen-free extract.

mixing. A meat grinder (Javar 32, JAVAR LTDA, Bogotá, Columbia) was used to pelletize the wet mixture. The 2 mm pellets were dried in a forced-air oven at 60 °C for 10 h. The pellets were packed in plastic bags and refrigerated at 10 °C until use. Diets and ingredients were analysed for chemical composition according to standards (AOAC 1995).

The pellet stability in water of each diet was determined at 2, 4 and 8 h following the method of Maguirre, Allan, Baigent and Frances (1988). Data were expressed as the percentage dry matter loss (DML) after the immersion period in seawater.

### Feeding trials

Juvenile white shrimp (0.35 ± 0.01 g) were obtained from a nursery facility for feeding trials conducted at the Centro de Obtención y Cría de Larvas, Santa Cruz del Sur, Cuba. The shrimp were acclimated to laboratory conditions for 48 h in a 1000-L tank. Triplicate sets of 40-L plastic containers (65 cm × 43 cm) were used for each diet. After acclimation, the organisms were randomly stocked at 10 juveniles per container.

Seawater (33.6 ± 0.76 g L$^{-1}$ salinity) was passed through sand (10 μm) and cartridge (5 μm) filters and a UV light unit before entering the tanks. Seawater was replaced daily at 30% of volume. Following recommendations for juvenile white shrimp (CIP 2003), the temperature was maintained at 28.3 ± 0.93 °C, pH 8.1 ± 0.09 and constant aeration with air stone diffusers to maintain dissolved oxygen at 5.2 ± 0.36 mg L$^{-1}$. Water temperature and dissolved oxygen were monitored twice a day using an oxygen meter (YSI-58, Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). Salinity was recorded with a refractometer (Atago 2401, Atago Co., Ltd, Tokyo, Japan). pH was measured with a pH meter (UC-12, JICC Co., Ltd, Tokyo, Japan) on a weekly basis. Photoperiod was set at a 12-h light:dark cycle. The feed trial for substituting soybean meal for fishmeal was run for 52 days.

Feed was offered in two equal portions at 09:00 and 16:00 hours to apparent satiation. At the end of the trial, each shrimp was individually weighed to the nearest 0.01 g using a digital balance. The final weight, survival, feed conversion ratio (FCR) and protein efficiency ratio (PER) were used to evaluate the effect of substitution in the diet, according to:

$$\mathrm{FCR} = A/B_{\mathrm{f}} - B_0$$
$$\mathrm{PER} = (B_{\mathrm{f}} - B_0)/P_{\mathrm{a}}$$

where $A$ is the feed added, $B_{\mathrm{f}}$ is the final biomass, $B_0$ is the initial biomass and $P_{\mathrm{a}}$ is the protein added.

### Statistical analysis

The normality and homogeneity of final weight, FCR and PER were determined with Kolmogorov–Smirnoff and Bartlett's tests. One-way ANOVA and Tukey's HSD test were used for finding significant differences. Optimal substitution level was estimated by the broken-line method (Shearer 2000). Significance was set at $P < 0.05$. The tests were run with the STATISTICA™ 6.0 software.

## Results

At the end of the experiment, shrimp weight varied from 0.64 to 1.06 g; the FCR varied from 2.8 to 7.9; and the PER varied from 0.45 to 1.21. Significant differences occurred as a consequence of the percentage of substitution in the diets (Table 3). Overall, better results were obtained with diets where soybean meal was substituted at levels up to 75% (S46, S59 and S75). The total replacement diet (S100) resulted in poor performance of shrimp. Final survival was acceptable for all treatments (90% or higher), with no significant differences between treatments.

Regression analyses using the broken-line method yielded significant results for final weight, FCR and PER (Fig. 1). Estimates of an adequate substitution level derived from the broken line were 74.6% for final weight, 76.5% for the FCR and 78.5% for the PER. Using the broken-line method, the estimates were consistent, indicating that 76.5 ± 2% substitution is optimal, which closely approximates the experimental diet S75.

Pellet stability in water was significantly related to the level of dietary soybean meal (Table 4). After 2 h of immersion, diets lower in soybean meal content

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**Table 3** Final weight, FCR and PER for juvenile *Litopenaeus schmitti* in relation to substitution of soybean meal for fishmeal*

| | Diet† | | | | |
|---|---|---|---|---|---|
| | S46 | S59 | S75 | S88 | S100 |
| Final weight | 1.06 ± 0.010[a] | 1.06 ± 0.006[a] | 1.07 ± 0.003[a] | 0.75 ± 0.012[b] | 0.64 ± 0.003[c] |
| FCR‡ | 3.0 ± 0.29[a] | 2.8 ± 0.13[a] | 2.8 ± 0.14[a] | 5.5 ± 0.15[b] | 7.9 ± 0.04 [c] |
| PER[3] | 1.01 ± 0.092[a] | 1.14 ± 0.060[a] | 1.21 ± 0.068[a] | 0.63 ± 0.017[b] | 0.44 ± 0.003[b] |

*Initial shrimp weight, 0.35 g.
†Means of triplicate samples ± SE. Values in the same row with different superscript letters are significantly different.
‡FCR, feed conversion ratio; PER, protein efficiency ratio.

(S46, S59 and S75) resulted in lower DML (14%). After an 8-h immersion, the effect of soybean content was more clearly established. Pellet stability progressively decreased as the soybean content increased. Dry matter loss reached 32% where soybean meal was included at a rate of 100% substitution (S100).

## Discussion

The results indicate that soybean meal is an adequate substitute for fishmeal in diets for juvenile white shrimp, with a substitution rate of 76.5 ± 2% maximizing growth response, feed usage and protein efficiency.

The growth rates observed in this study are consistent with those reported by other authors working with juvenile *L. schmitti* in clear water. Fraga, Galindo, Reyes, Alvarez, Gallardo, Forrellat and González (1996) evaluated different protein sources for juveniles (0.4 g) and obtained final weights within 0.79–1.07 g after 49 days. This is similar to the growth rate and maximum final weight observed in this study. Galindo, Alvarez, Fraga, Reyes, Jaime and Fernández (1992) determined lipid requirements for juveniles (0.75 g) and, after 47 days, the final weight was within 0.81–1.54 g. Galindo (2000) evaluated different protein sources for juveniles (0.25 g) and, after 52 days, obtained final weights within 0.65–0.8 g. The author tested yeast, sesame seed, shrimp, sunflower and soybean meals, and found that the best growth, FCR, and PER were obtained with soybean meal. The growth rates of juvenile *L. schmitti* are lower than those of juvenile *L. vannamei* (Davis, Arnold & McCallum 2002), which is currently used for commercial aquaculture operations.

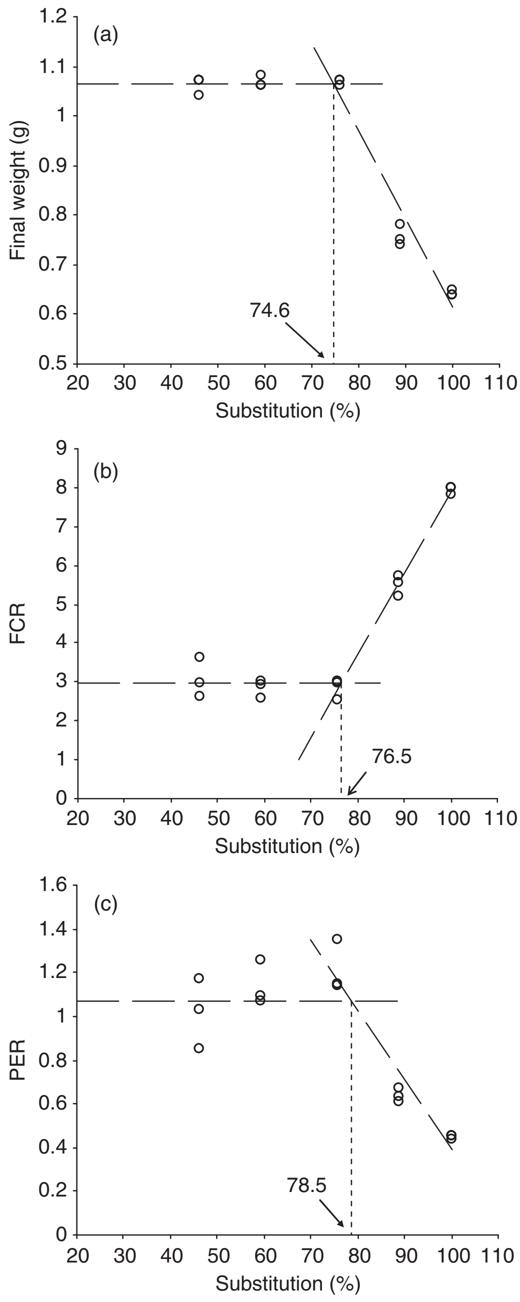


**Figure 1** Effect of substituting soybean meal for fishmeal on final weight (a), feed conversion ratio (FCR, b) and protein efficiency ratio (PER, c) of juvenile white shrimp *Litopenaeus schmitti*.

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**Table 4** Pellet stability in seawater as per cent dry matter loss in diets with soybean meal substituted for fishmeal at different immersion times

| | Soybean meal substitution diet* | | | | |
|---|---|---|---|---|---|
| Time (h) | S46 | S59 | S75 | S88 | S100 |
| 2 | $14.25 \pm 0.06^{a}$ | $14.29 \pm 0.09^{a}$ | $14.37 \pm 0.10^{a}$ | $20.32 \pm 0.13^{b}$ | $22.04 \pm 0.08^{c}$ |
| 4 | $16.93 \pm 0.13^{a}$ | $17.01 \pm 0.11^{a}$ | $17.05 \pm 0.08^{a}$ | $24.76 \pm 0.11^{b}$ | $27.45 \pm 0.17^{c}$ |
| 8 | $20.03 \pm 0.16^{a}$ | $20.62 \pm 0.07^{b}$ | $22.12 \pm 0.20^{c}$ | $29.47 \pm 0.43^{d}$ | $32.53 \pm 0.48^{e}$ |

*Means of triplicate samples ± SE. Values in the same row with different superscript letters are significantly different.

Soybean meal is considered to be a good substitute for fishmeal in diets for other shrimp species (Pascual *et al.* 1990; Akiyama, Dominy & Lawrence 1992; Davis & Arnold 2000; Mendoza-Alfaro *et al.* 2001). Gaxiola, García, Jaime and Gonzalez (1996) reported that the growth and survival of *L. schmitti* postlarvae improved when they were fed diets containing a fishmeal: soybean meal ratio of 0.6:1. This ratio is within the range 1.16:1–0.31:1 (diets S46, S59 and S75) producing better response of juvenile *L. schmitti*. High substitution levels are possible for white shrimp, as it has shown in the wild to be herbivorous, rather than carnivorous (Anderes 1982, 1984). This is related to the digestive enzyme activity in the species. The *in vitro* digestibility of soybean meal by white shrimp was higher (83.2%) than when other protein sources (70.5%), such as fishmeal, were used (Carrillo 1994).

The experimental diets with substitutions above 75% resulted in diminished performance. Lower final weight, FCR and PER occurred with a 100% substitution (54% soybean meal) diet. Soybean meal above 50% in diets for crustaceans and penaeid species was discouraged because of low palatability and pellet stability (Akiyama 1990; Lim & Dominy 1992; Floreto *et al.* 2000).

Lawrence *et al.* (1986) found that animal protein sources could be included at levels as low as 7% in shrimp diets and recommended 40–50% soybean levels. However, these authors included shrimp-head meal in their test diets, which is an attractant that may also improve diet palatability. In our study, fishmeal was the only animal protein source, thus limiting the substitution potential. Shifts in nutrient profiles and concentration levels on the experimental diets were a consequence of a design that required the evaluation of shrimp response to variations in fish and soybean meal combinations for practical formulation purposes.

Soybean meal can produce poor diet palatability (Webster, Tidwell, Goodgame, Yancy & Mackey 1992; Liener 1994; Forster *et al.* 2002; Thiessen, Campbell & Tyler 2003). High dietary concentrations of soybean products negatively affect palatability in some species of shrimp. For example, Lim and Dominy (1990) reported a significant decline in feed intake of *Penaeus vannamei* with dietary soybean meal concentration exceeding 42%. This may be the cause of the poor results we obtained with diets containing more than 41% soybean meal. Additionally, lower pellet stability at higher concentrations of soybean meal could result in diminished nutritional value caused by leaching. After 2 h of immersion in water, diets lost more than 20% dry matter. Cruz-Suárez (2004) reported that shrimp feeds with a DML over 20% after a 2-h immersion should be considered inadequate. This may be associated with the high fibre content in diets S88 and S100 (4.1% and 4.3% respectively). Some researchers recommend that total fiber content should not exceed 4% because a higher fibre content increases pellet DML and faecal production, reduces diet digestibility and digestive enzyme efficiency and, in general, lowers diet nutritional quality (Akiyama *et al.* 1992; Velasco 2002).

It can be concluded that substituting up to 76.5 ± 2% soybean meal for fishmeal is feasible and can benefit the growth response of juvenile white shrimp.

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