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Substituting fishmeal with mixtures of wheat, corn and soya bean meals in diets for the white leg shrimp, *Litopenaeus vannamei* (Boone): effect on production parameters and preliminary economic assessment

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Abstract

We analysed the effect on production and economic performance of juvenile *Litopenaeus vannamei* of isoproteic diets substituting fishmeal by 25%, 50%, 75% and 100% with mixtures of wheat, soya bean and cornmeals. In a laboratory trial, 10 juveniles m^{-2} (1.1 ± 0.1 g) were reared in 60-L plastic containers using a recirculation system for 90 days. Three replicates were used to test each diet. A commercial diet serving as a reference and the diet with the highest content of essential amino acids (50% substitution, 6.46 ± 1.1 g) produced significantly higher shrimp final weight (7.12 ± 0.9 g, $P < 0.05$). There were not significant differences in specific growth rate and mean survival (85.9 ± 0.2%, $P > 0.05$). In a pond trial, 10 shrimp m^{-2} (1.08 ± 0.3 g) were cultivated in 1.5 m^{-3} cages for 35 days, testing the diets in triplicate. Final weight was significantly higher ($P < 0.05$) when 100% substitution was used (10.89 ± 0.24 g), while survival did not differ significantly among diets ($P > 0.05$). The optimal level of substitution was estimated at 86.0%. Apparently, nutrients contained in the diets combined well with natural feed available in the pond, up to a point where 7.3% of fishmeal inclusion is recommended. An economic analysis showed that 100% substitution produced the best results. We conclude that mixtures of wheat, corn and soya meals are potential alternatives to replace fishmeal effectively in diets for *L. vannamei*.

Keywords: plant proteins, shrimp nutrition, fishmeal replacement, shrimp growth

Introduction

The increasing demand of high-quality protein for animal and human consumption, together with the variability in fish landing associated with El Niño events, has increased the demand and price of fishmeal for aquafeed preparation (Hardy 2010).This situation has led feed producers to seek for cheaper alternative protein sources that could contribute to reducing feed costs, such as plants containing high-quality protein.

The nutritional requirements of the Pacific white leg shrimp *Litopenaeus vannamei* (Boone) have been studied for several cultivation conditions (Cuzon, Lawrence, Gaxiola, Rosas & Guillaume 2004; NRC 2011; Li, Xu, Qin & Chen 2015). In general, shrimp diets contain from 25% to 50% of fishmeal, representing the most expensive protein ingredient (Amaya, Davis & Rouse 2007a), affecting economic profitability. Much research has been focused on the use of marine by-products, meat and bone meal, poultry and agriculture by-products that effectively replace or minimize the inclusion of fishmeal in commercial shrimp diets (Davis & Arnold 2000; Samocha, Davis, Saoud & DeBault 2004; Forster, Dominy, Conquest, Ju & Grey 2010).

Although omnivorous, *L. vannamei* requires a high-protein diet (Goytortúa-Bores, Civera-Cerecedo, Rocha-Meza & Green-Yee 2006; Suárez, Gaxiola, Mendoza, Cadavid, García, Alanis, Suárez, Faillace & Cuzon 2009), either under intensive or laboratory culture conditions. For instance, Venero, Davis and Rouse (2007) obtained the highest growth feeding *L. vannamei* with a diet containing 40% crude protein, compared with other diets using lower crude protein concentrations. Gauquein, Cuzon, Gaxiola, Rosas, Bureau and Cochard (2007) and Amaya, Davis and Rouse (2007b) reported similar results for the same species testing diets with a crude protein content ranging from 25% to 58%.

There are several reports on partial replacement of fishmeal with plant proteins in diets for various shrimp species (Lim & Dominy 1990; Sudaryono, Hoxey, Kailis & Evans 1995; Álvarez, Hernández-Llamas, Galindo, Fraga, García & Villarreal 2007; Suárez *et al.* 2009). In those studies, the dietary inclusion of, for example, soya bean meal up to 58% did not adversely affect shrimp production. Other plant sources such as cottonseed, canola and legume meals have also been used (Li, Robinson & Hardy 2000).

It is accepted that using a plant protein source alone could only partially replace fishmeal in crustacean diets due to its deficiency in essential amino acids, the presence of anti-nutritional factors, poor palatability and lower utilization of nutrients (Davis & Arnold 2000; Samocha *et al.* 2004; Tantikitti 2014). A mixture of plant protein sources could improve the amino acid profile in practical diets (Amaya *et al.* 2007b).The appropriate combination of cereals and legumes enables the complementation of limiting amino acids in formulated diets (Mensa-Wilmot, Phillips & Hangrove 2001). For instance, the use of complex mixtures of plant protein sources has been found to significantly improve Nile tilapia (*Oreochromis niloticus*, Linnaeus) production (Borgeson, Racz, Wilkie, White & Drew 2006).

There is limited knowledge on the replacement of fishmeal with a mixture of plant protein sources for the Pacific white leg shrimp. Particularly, no studies have been conducted reporting the feasibility of improving shrimp growth, production and economic performance when substituting fishmeal by mixtures of plant meals. The aim of this study was to determine the effect on production and economic performance of juveniles *L. vannamei* of isoproteic diets partially and entirely replacing fishmeal with mixtures of wheat, soya bean and cornmeals. No antecedents were found in the literature evaluating, from the zootechnical and economic perspective, the use of such cereal mixture as a substitute of fishmeal in shrimp diets.

Materials and methods

Experimental animals

Shrimp were obtained from the stock of the Centro Interdisciplinario de Investigación y Desarrollo Integral Regional (CIIDIR-IPN), Unidad Sinaloa, México. Juveniles *L. vannamei* were collected from a broodstock tank and sorted to homogenize shrimp size.

Diet formulation and preparation

Following the method described by Houser and Akiyama (1997), five diets were formulated from practical and semi-purified ingredients to contain 32% of crude protein and 9.5% lipids. The diets included 0%, 25%, 50%, 75% and 100% fishmeal substitution by mixtures of corn, wheat and soya bean meals. The plant meals were mixed in amounts necessary to produce isoproteic and isolipidic diets (Table 1). A 35% crude protein shrimp commercial diet (Azteca ®, Guadalajara, Jal., Mexico) served as a reference diet.

Feedstuffs were ground (Grindmaster, Model 505, Louisville, KY, USA) and sieved (No. 60, FIIC, SA de CV, MX) through a 100-µ sieve. A domestic blender (KitchenAid, Model 600; Benson Harbor, MI, USA) was used to mix the ingredients for 30 min, and fish oil and soya lecithin were added gradually to the dry mix. Water was added to make a stiff dough. The wet mixture was pressure pelleted through a meat grinder (Tororey®, Monterrey, NL, Mexico), steamed for 5 min and dried in a draft oven at 60°C overnight, or until pellets contained around 10% moisture. Finally, diets were broken at 3–4 mm size and stored in plastic bags at 5°C until use.

Table 1 Formulation and chemical composition of diets for shrimp *Litopenaeus vannamei* (Boone) using 0%, 25%, 50%, 75% and 100% substitution of fishmeal by mixtures of plant (wheat, corn, and soya bean) meals

	Substitution (%)				
Ingredient (%)	0	25	50	75	100
Fishmeal	52.0	39.1	26.0	13.0	–
Cornmeal	–	9.5	21.0	21.0	18.0
Wheatmeal	–	9.5	20.0	19.0	21.0
Soya bean meal	–	9.5	20.0	34.0	48.0
Cellulose	35.0	19.4	–	–	–
Binder (grenetine)	4.0	4.0	4.0	4.0	4.0
Fish oil	4.0	4.0	4.0	4.0	4.0
Soya bean lecithin	4.0	4.0	4.0	4.0	4.0
Vitamins and mineral premix†	1.0	1.0	1.0	1.0	1.0
Proximate composition (%)					
Moisture	7.59 ± 0.14	7.85 ± 0.05	8.27 ± 0.07	8.35 ± 0.47	8.26 ± 0.25
Protein	32.02 ± 0.22	31.92 ± 0.12	32.88 ± 0.30	32.62 ± 0.20	32.46 ± 0.24
Lipids	9.85 ± 0.03	9.81 ± 0.23	10.17 ± 0.03	9.42 ± 0.12	9.11 ± 0.22
Crude fibre	7.22 ± 0.23	7.00 ± 0.11	6.72 ± 0.12	6.55 ± 0.09	6.30 ± 0.11
Ash	0.29 ± 0.04	0.66 ± 0.06	1.14 ± 0.08	1.57 ± 0.06	1.94 ± 0.02
Nitrogen-free extract	50.62	50.60	49.08	49.83	50.18

†Vitamins and mineral premix: retinol, 5000 IU; cholecalciferol, 4000 UI; α-tocopherolacetate, 100 mg kg^{-1}; menadione, 5 mg kg^{-1}; thiamin, 60 mg kg^{-1}; riboflabin, 25 mg kg^{-1}; pyridoxine HCL, 50 mg kg^{-1}; pantothenicacid, 75 mg kg^{-1}; niacin, 40 mg kg^{-1}; biotin, 1 mg kg^{-1}; inositol, 400 mg kg^{-1}; cyanocobalamin, 0.2 mg kg^{-1}; folic acid, 10 mg kg^{-1}. KCl, 0.5 g kg^{-1}; $MgSO_4 \cdot 7H_2O$, 0.5 g kg^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.09 g kg^{-1}; $MnCl_2 \cdot 4H_2O$, 0.0234 g kg^{-1}; $CuSO_4 \cdot 5H_2O$, 0.005 g kg^{-1}; KI, 0.0005 g kg^{-1}; $CoCl_2 \cdot 2H_2O$, 0.00025; Na_2HPO_4, 2.37 g kg^{-1}.

Chemical composition

Three samples from each diet were pooled and analysed for proximate composition (Table 1). Crude protein was determined using the Kjeldahl method (APHA 1989), and crude lipid was extracted with anhydrous ether in a Soxtec (Bligh & Dyer 1959). Ash was determined using a muffle furnace (AOAC 2000), crude fibre with the phenol-sulphuric acid method (Myklestad & Haug 1972), and the nitrogen-free extract was obtained by difference. The amino acid content of tested diets (Table 2) was determined based on Tacon, Webster and Martínez (1984).

Laboratory trial

Juveniles (0.92 ± 0.03 g) were randomly selected and stocked at 10 juveniles m^{-2} in 60-L plastic tanks in an indoor laboratory at CIIDIR-IPN. Diets were tested in triplicate by randomly assigning them to the tanks. Water exchange (50%) in the tanks was performed daily using a recirculation system. Water temperature was maintained at 28.0 ± 1°C introducing an aquarium heater (100-W) into the water inlet container. Each tank was permanently aerated using a diffuser stone connected to a 2-Hp blower. Dissolved oxygen was 4.0 ± 0.5 mg L^{-1}, and pH varied within 7.66–7.98 throughout the experiment. Mean salinity was 35.0 ± 0.5 ups. Previous to the trial, acclimation was carried out for seven days under the same conditions used for the assay.

Table 2 Amino acid composition of the experimental diets (g kg^{-1}) substituting fishmeal with a mixture of plant (wheat, corn and soya bean) meals

	Substitution (%)				
Amino acid	0	25	50	75	100
Arginine	16.9	20.4	25.4	26.0	25.4
Cysteine	4.0	5.6	7.9	7.9	7.3
Methionine	10.1	11.0	12.8	11.1	8.3
Threonine	14.0	15.8	18.8	17.5	15.1
Isoleucine	16.1	18.2	21.8	20.7	18.4
Leucine	23.0	34.7	50.9	49.5	42.8
Lysine	28.9	26.6	25.3	21.9	17.9
Valine	18.9	21.5	25.6	24.0	20.8
Tyrosine	11.9	15.5	20.8	19.7	16.6
Tryptophan	3.0	3.1	3.2	3.3	3.5
Phenylalanine	12.2	16.6	22.9	22.6	20.4
Histidine	9.8	11.4	13.9	13.0	11.1

Temperature, dissolved oxygen and salinity were monitored daily with a digital thermometer, an oxygen meter (model 55; YSI, Yellow Springs, OH, USA) and a hand refractometer (model 300011; Sper Scientific, Scottsdale, AZ, USA) respectively. Photoperiod (12 L:12 D) was maintained with artificial light (39 watts daylight bulbs). Shrimp were fed twice daily (09:00 and 17:00 h), and faeces and feed waste were siphoned daily. A feed ration of 10% shrimp biomass was initially used, and subsequently, the ration was adjusted depending on the amount of feed residues. Shrimp were fed with the experimental and reference diets for one week before starting the experiment, which lasted 90 days.

Pond trial

Eighteen cages of 1 m^3 (1 m × 1 m × 1 m) made of plastic mesh (500 μm) were placed into a 3.5 ha pond at the Acuícola Camaronera Styl semi-intensive farm (Ejido La Culebra, Sinaloa, México). The cages were suspended using poles allowing for a separation of 10 cm between their bottom and pond's bottom. The cages were placed in a row with one-metre separation between them at the centre of the pond and, to homogenize water exchange, the row of cages was set perpendicular to the main water flow in the pond. Diets were tested in triplicate by randomly assigning them to the cages. Juveniles (1.08 ± 0.3) were stocked at 10 shrimp m^{-2}. Water temperature (30 ± 1.5°C) and dissolved oxygen (3.2 ± 0.5 mg L^{-1}) were measured with an oxygen meter (YSI® Model 55), and salinity (38.0 ± 0.5 ups) was measured with a hand refractometer (Sper Scientific®). Water parameters were monitored weekly. Cages were cleaned every three days with a brush. Shrimp were fed twice daily (09:00 and 17:00 h). A 10% feed ration of shrimp biomass was initially used, and subsequently, the ration was adjusted depending on feed consumption. Feeding trays (20 cm diameter, 2 mm bottom mesh) containing 30% of the feeding ration were used to monitor daily consumption. Shrimp were fed with the experimental and reference diets for one week before starting the experiment, which lasted 35 days.

Growth analysis

Shrimp weight (g) was measured every two weeks in the laboratory trial and every week for the pond assay. In the case of the laboratory trial, the specific growth rate (SGR) was calculated following Hopkins (1992), accordingly:

$$\mathrm{SGR} = (\mathrm{Ln}\, w_f - \mathrm{Ln}\, w_i)/t \qquad (1)$$

where w_f and w_i are final and initial weight, and t is the time (days) used for the growth trial. Following the recommendation by Hopkins (1992), SGR was not reported as a percentage.

The growth curves observed for the pond trial were sigmoidal in shape, rather than exponential (i.e. the basic assumption underlying the estimation of SGR), and after Hopkins (1992), the SGR was not calculated. Instead, the following equation was used (Ruiz-Velazco, Hernández-Llamas, Gomez-Muñoz & Magallon 2010):

$$w_t = w_i + (w_f - w_i)\left(\frac{1-k^t}{1-k^{t_f}}\right)^3 \qquad (2)$$

where k is a growth coefficient and t is the number of units of time for which w_t is predicted (e.g. 20, if w_t is predicted for 20 days) and t_f is the time at the end of the growth trial.

Economic analysis

A preliminary study was conducted to explore the economic feasibility of substituting fishmeal with the mixtures of plant meals using the results from the pond trial. For this purpose, the meal prices ton^{-1} in the international market (USD$) were used, and economic income was calculated using the production results obtained with the pond trial and the price of shrimp in Mexico. Typically, shrimp is sold according to a farm-gate 'basic' price, and the actual sale price is calculated by 'adding' to the 'basic' price the mean individual weight of shrimp. For example, if the 'basic' price is MX$40.0 kg^{-1} and the mean weight of shrimp is 10 g, the sale price is MX$50.0 kg^{-1}. The 'basic' price was taken from CLUSAC (2015) where the minimum commercial weight of shrimp is established at 10 g. For every experimental diet, net income was calculated by subtracting the total cost associated with fishmeal and the mixture of plant meals from the economic income.

It must be noted that this preliminary analysis does not pretend to project the actual economic performance of culture operations using the diets as there are other costs not considered for analysis, such as those of other feed ingredients and manufacturing, and operating costs like seed, energy and labour. Instead, the purpose of the study was limited to provide an economic framework for comparison of the experimental diets.

Statistical analysis

The data were tested for normality (Lillifors' test) and heteroscedasticity (Bartlett's test). Differences between final weights were determined by analysis of variance and a Tukey's test. Arcsin transformations were applied to percentages before the analysis (Zar 2010). The statistical analysis was carried out using Jandel Sigmastat statistical package version 2.0. Invariance tests (Ratkowsky 1983) were conducted to detect differences in values of the growth parameters in Eqn 2 among the diets. Nonlinear regression procedures available in GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) were used for this analysis.

Following Shearer (2000) and Hernández-Llamas (2009), a second-order polynomial, the 'broken-line' and the 'broken-curve' models were compared for relating the final weight of shrimp and percentage of fishmeal substitution and determine the optimum level of replacement. As the models contain a different number of parameters, they were compared regarding residual variance to test model parsimony, avoiding over-parameterization (Hernández-Llamas 2009). Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA) was used for model comparison. The polynomial equation yielded the best result, and a lack-of-fit test was conducted to assess its adequacy, using commands in Stata 13.0 (StataCorp LP, College Station, TX, USA). Accordingly, a non-significant outcome of the test indicates a satisfactory fit of the model (Zar 2010). The significance level was set at $P < 0.05$ for all the analyses.

Results

There were significant differences in final weight among the diets in the laboratory trial (Table 3). The reference diet yielded a significantly higher final weight (7.12 ± 0.9) compared with the experimental diets, except the diet using 50% substitution which did not differ significantly from the reference diet. No significant differences were detected in SGR, which varied from 0.0226 ± 0.012 (reference diet) to 0.0201 ± 0.011 (0% substitution diet). No significant differences were found in survival among the treatments, averaging 84.1 ± 0.86%.

The invariance tests using Eqn 2 showed that there were significant differences in final weight of shrimp between 100% substitution diet and 50%, 25% and 0% replacement diets in the pond trial, and there were no significant differences in the growth coefficient among the diets (Table 4, Fig. 1). The highest mean final weight (10.89 ± 0.24 g) was obtained with the 100%

Table 3 Growth and survival of shrimp *Litopenaeus vannamei* (Boone) after 90 days in the laboratory trial receiving diets with increasing substitution levels of fishmeal by a mixture of plant (wheat, corn and soya bean) meals, and a reference diet

Substitution (%)	Initial weight (g)	Final weight (g)	SGR	Survival (%)
0	0.98 ± 0.2a	6.09 ± 0.5b	0.0201 ± 0.001a	83.3 ± 0.0a
25	0.91 ± 0.3a	6.25 ± 0.7b	0.0214 ± 0.008a	83.3 ± 0.0a
50	0.95 ± 0.2a	6.46 ± 1.1ab	0.0212 ± 0.004a	83.3 ± 0.0a
75	0.93 ± 0.2a	5.75 ± 0.7b	0.0201 ± 0.014a	88.5 ± 0.5a
100	0.88 ± 0.1a	6.38 ± 0.8b	0.0218 ± 0.008a	83.3 ± 0.0a
Reference	0.92 ± 0.2a	7.12 ± 0.9a	0.0226 ± 0.012a	83.3 ± 0.0a

Mean values sharing a common letter are not significantly different ($P < 0.05$)

Table 4 Growth and survival of shrimp *Litopenaeus vannamei* (Boone) after 35 days in the pond trial receiving diets with increasing substitution levels of fishmeal by a mixture of plant (wheat, corn and soya bean) meals and a reference diet

Substitution (%)	Initial weight (g)	Final weight (g)	Growth coefficient (*k*)	Survival (%)
0	1.02 ± 0.06a	8.96 ± 0.55b	0.956 ± 0.013a	96.66 ± 5.7a
25	1.09 ± 0.11a	9.83 ± 0.51b	0.947 ± 0.011a	100.0 ± 0.0a
50	1.10 ± 0.10a	10.60 ± 0.16b	0.942 ± 0.003a	96.66 ± 5.7a
75	1.06 ± 0.12a	10.61 ± 0.27ab	0.939 ± 0.005a	100.0 ± 0.0a
100	1.13 ± 0.12a	10.89 ± 0.24a	0.946 ± 0.004a	93.33 ± 5.7a
Reference	1.10 ± 0.10a	10.06 ± 0.52b	0.948 ± 0.011a	100.0 ± 0.0a

Mean values sharing a common letter are not significantly different ($P < 0.05$)

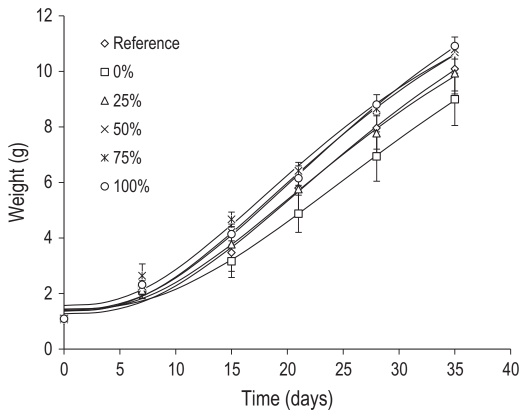

Figure 1 Growth of *Litopenaeus vannamei* (Boone) in the pond trial receiving diets with different substitution percentages of fishmeal by mixtures of wheat, corn and soya bean meals and a reference diet.

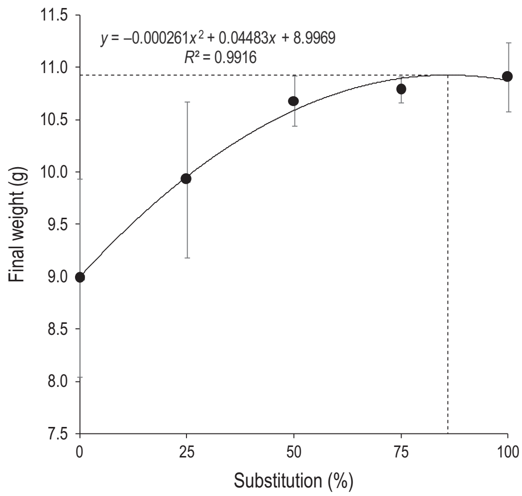

Figure 2 Relationship between the final weight of shrimp and percentage of fishmeal substitution by wheat, corn and soya bean meals in the pond trial. The dashed line indicates the replacement level (86%) where the maximum final weight (10.92 g) can be obtained.

substitution diet. Final survival did not differ significantly among the diets (mean = 97.75%).

A lack-of-fit test showed that the second-order polynomial satisfactorily fits the curve relating shrimp final weight and percentage of fishmeal substitution ($P > 0.96$). According to this equation, the maximum final weight (10.92 g) would be obtained by 86.0% replacement (i.e. 7.3% inclusion of fishmeal, Fig. 2).

The economic analysis allowed determining that there were significant differences in the total production cost associated with the ingredients, the production cost necessary to obtain one ton of shrimp and net income (Table 5). The minimum total cost (USD$ 572.1 ha^{-1}) and maximum net income (USD$ 2747.3 ha^{-1}) corresponded to the 100% substitution diet, while the maximum total cost (USD$ 2241.0 ha^{-1}) and minimum net income (USD $486.0 ha^{-1}) corresponded to the diet without replacement (Table 5).

Discussion

The results show that mixtures of wheat, corn and soya meals can be used to partially or totally substitute fishmeal as a protein source in diets for *L. vannamei*. Moreover, using those mixtures under pond culture conditions did not impair shrimp growth, but improved it, as well as production and economic performance.

The shrimp growth observed in the pond trial was typical of shrimp offered a high-quality, practical diet under research conditions (Samocha *et al.* 2004; Hernández, Olvera-Novoa, Aguilar-Vejar, González-Rodríguez & Abdo de la Parra 2008). Survival in the pond trial was high, indicating good health condition of shrimp, the adequacy of water quality parameters and absence of nutrient deficiency in the diets. In general, survival of shrimp was high for all treatments, and there were no indications of the feed being rejected or inadequate in palatability. Samocha *et al.* (2004) reported similar growth and survival of *L. vannamei* when entirely replacing fishmeal with a mixture of extruded soya bean and poultry by-product meal supplemented with egg.

The results from the laboratory trial suggest that fishmeal can be wholly substituted with the tested ingredients without adverse effects on shrimp performance. The SGR was similar to 0.019 reported by Davis and Arnold (2000) (value divided by 100 for comparison) for *L. vannamei* reared for 42 days using co-extruded soya bean/poultry by-product meal in a practical diet, but lower than 0.039 reported by Suárez *et al.* (2009) (value divided by 100 for comparison) for the same species when fishmeal was replaced with soya and canola meals. We observed a favourable response of shrimp to the 50% substitution diet, possibly as a consequence of its amino acid content. The amino acid profile in this diet, although low compared with the recommended dietary level for *L. vannamei* (Akiyama, Dominy & Lawrence

Table 5 Mean values of variables of preliminary economic analysis of diets for shrimp *Litopenaeus vannamei* (Boone) with different substitution levels of fishmeal by a mixture of plant (wheat, corn and soya bean) meals

Substitution (%)	FCR†	SP‡	TF§	CITF¶	TCI††	PCTS‡‡	SP§§	I¶¶	NI†††
0	2.08	0.87	1.80	1242.0	2241.0a	2587.6a	3.14	2727.3	486.0a
25	2.15	0.98	2.11	1007.2	2130.7ab	2167.5b	3.20	3147.4	1016.7b
50	2.11	1.02	2.16	783.8	1692.5bc	1651.8c	3.24	3328.4	1635.9c
75	2.06	1.06	2.18	529.8	1156.0c	1089.6d	3.24	3447.3	2291.2d
100	2.03	1.02	2.06	276.7	572.1c	562.9e	3.26	3319.5	2747.3d

The costs per ton of the ingredients in the international market are (USD$): fishmeal (2388.5); cornmeal (178.67); wheatmeal (200.34); and soya bean meal (418.09) (the World Bank, http://www.worldbank.org/). Production data from Table 4 were used for calculation. Mean values sharing a common letter are not significantly different ($P < 0.05$).

†Feed conversion ratio.

‡Estimated shrimp production (ton ha^{-1}).

§Tons of feed required for SP.

¶Cost of ingredients ton^{-1} of feed based on the international prices and the formulation in Table 1.

††Total cost of ingredients necessary to obtain SP.

‡‡Production cost of ingredients ton^{-1} of shrimp.

§§Shrimp price kg^{-1}. Prices were calculated assuming shrimp could be sold, despite shrimp did not reach minimum commercial weight (10 g) with diets using 0% and 25% fishmeal substitution.

¶¶Income ha^{-1}.

†††Net income above TCI ha^{-1}.

1999), contained essential amino acids such as methionine, threonine, isoleucine, leucine, phenylalanine and histidine in higher concentrations compared with the rest of the experimental diets.

The results from the pond trial showed it is feasible to substitute fishmeal with the tested ingredients partially. The average growth rate in this trial ranged from 1.58 g $week^{-1}$ (no replacement diet) to 1.95 g $week^{-1}$ (complete substitution diet), which is comparable to 1.79 g $week^{-1}$ obtained with the commercial diet serving as a reference, and acceptable for semi-intensive commercial farming of *L. vannamei*. From Table 4, we estimated that increasing substitution from 0% to 100% results in improving the final weight of shrimp in 17%. It must be noted, however, that as explained for Fig. 2, using 86.0% substitution these results could be slightly enhanced.

Amaya *et al.* (2007a) used ponds to test the replacement of 0%, 3%, 6% and 9% fishmeal with a mixture of soya bean and corn gluten meal in practical diets for *L. vannamei* and did not find significant differences in production parameters and gross economic returns (income – total feed inputs costs), possibly as a consequence of the low levels of substitution used. In our study, results from the economic assessment indicated that increasing replacement from 0% to 100% resulted in a decrease of 78.2% in production cost of one ton of shrimp and an increase in net income of 465%. Clearly, increasing replacement has a much more significant effect on improving economic efficiency than on production efficiency. It is conceivable that increasing substitution could result in differences in shrimp flesh quality. However, our objective was to make a preliminary economic evaluation of diets, and further research should be conducted testing the effect of substitution on flesh quality.

Some studies, based on non-significant results, suggest that fishmeal can be entirely substituted with alternative ingredients in diets for *L. vannamei* (Amaya *et al.* 2007a; Bauer, Prentice-Hernandez, Borges-Tesser, Wasielesky & Poersch 2012). In contrast to what has been reported by those authors, in the pond trial, we found that replacing fishmeal did not impair shrimp growth but significantly improved it up to 86% substitution. To our knowledge, there are no antecedents of investigations reporting that growth of *L. vannamei* can be significantly improved when substituting fishmeal with plant ingredients. According to our results, 7.3% inclusion of fishmeal was optimal for shrimp growth in the pond trial, closely approximating the low fishmeal inclusion levels of 7.5–12.5%, reported by Fox, Lawrence and Smith (2004) as not compromising shrimp performance. It must be noted, however, that the results from our economic assessment suggest 7.3% is not necessarily the optimum from the economic perspective.

We used a second-order polynomial to estimate the response of final weight to percentage substitution because it is one of the most frequently used dose–response models in aquaculture nutrition, and it showed to fit better than the 'broken-line' and 'broken-curve' models (Shearer 2000; Hernández-Llamas 2009). This polynomial can describe curves with maxima and minima, and results from a lack-of-fit test indicated that the equation fitted adequately final weight data, with a maximum growth response occurring at 86.0% substitution.

Velasco and Lawrence (2000) and Tacon, Cody, Conquest, Divakaran, Foster and Decamp (2002) mentioned that the ability of shrimp to consume microbial organisms and organic matter produced in a pond, directly affect their growth and feed utilization, compared with a clear water culture system. It is well accepted that studies evaluating the ability of shrimp to utilize diets with low levels of fishmeal in clear water systems, significantly differ from those carried out in ponds (D'Abramo & Castell 1997). Results from clear water systems are thus of limited practical application unless they are validated under natural conditions in ponds (Moss & Divakaran 1995).

The use of net cages in ponds is useful to conduct an experiment under a realistic environment while keeping the assay on a small scale (Moss, Divakaran & Kim 2001). In our study, there was a better performance of shrimp reared in cages compared with tanks in the laboratory. Apart from the nutritional content of the tested diets, part of the success of substituting fishmeal with the plant-based protein sources in the pond was most likely a consequence of the possibility of shrimp of feeding on the natural feed existing in the pond (Leber & Pruder 1988). The walls and bottom of the cages we used were made of plastic mesh and, although walls were cleaned every three days to prevent fouling, we did not discard the possibility of shrimp feeding on organisms attached to the walls, and particularly the bottom, which was not cleaned. Further research is needed to study this possibility.

For our study, we used cellulose rather than a plant meal because we were testing the substitution of fishmeal with a mixture of plant meals. It is known that the digestibility of cellulose is very low (Akiyama, Coelho, Lawrence & Robinson 1989) and, while this could result in delaying stomach emptying, it is also known that it contributes to the efficient utilization of dietary protein (Gomez Diaz & Nakagawa 1990; Velurtas, Diaz, Fernández-Gimenez & Fenucci 2011). Guo, Liu, Tian and Huang (2006) reported that protein digestibility did not change with the levels of cellulose between 51.9 and 301.9 g kg^{-1}. The authors suggest this is probably a consequence of the diets used in their study contained adequate levels of protein, lipid and carbohydrate that were suitable for *L. vannamei*, further bringing about a preferential protein utilization of shrimp, which coincides with conclusions of Borer and Lawrence (1989). In our study, except 100% replacement in the pond trial, the results from ANOVA for both assays do not indicate that the diets incorporating cellulose resulted in significantly lower shrimp growth (Tables 3 and 4).

It is well known that the presence of anti-nutritional factors in plant protein impairs the growth of aquaculture species (Glencross, Booth & Allan 2007). In general, the presence of anti-nutritional factors in untreated foodstuffs usually results in loss of appetite, reduced growth and poor feed efficiency when used at high dietary concentrations (Tacon 2002). Strategies to overcome this limitation include using a complex mixture of plant protein sources, rather than one or two ingredients to reduce the exposure to individual anti-nutritional factors (Borgeson *et al.* 2006). Increasing diet complexity appears to be a feasible method for replacing fishmeal in aquaculture diets (Borgeson *et al.* 2006). In our study, natural feed in the pond apparently combined well with nutrients in diets containing a complex mixture of plant protein sources, fulfilling nutritional requirements of shrimp up to a point where a small 7.3% of fishmeal inclusion is recommended. Further research is needed for better understanding the contribution of natural feed in ponds to shrimp nutrition.

We conclude that mixing of wheat, corn and soya meals is a potential alternative to substitute fishmeal in practical diets for *L. vannamei* effectively. As previously indicated, the results of the economic assessment carried out in this investigation do not indicate the ultimate economic performance of the diets, yet it clearly shows the convenience of further investigating the use of substituting meals in practical diets for the species.

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