





Original Research Articles

Substituting fishmeal with extruded cull chickpea meal in diets for the white leg shrimp (*Litopenaeus vannamei*, Boone): A preliminary study of the effect on production parameters

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Keywords: Diets, chickpea, extruded feed, shrimp, *Litopenaeus vannamei*

<https://doi.org/10.46989/001c.87519>

Israeli Journal of Aquaculture - Bamidgeh

Vol. 75, Issue 2, 2023

Among the most typical feed ingredients for shrimp, plants represent a low-cost source in substituting for traditional high-cost feed ingredients. Extrusion is a common grains processing technique to make plant nutrients available and more digestible to animal. Different levels (15, 30, 45, and 60%) of extruded low-quality chickpea meal were included in a formulated diet for the juvenile shrimp *Litopenaeus vannamei* cultured in a closed system for 75 days. The growth of shrimp fed with the tested diets was similar to the control group ($P > 0.05$). We concluded that the use of extruded cull chickpea meal is a potential food alternative to replace fishmeal effectively in diets for *L. vannamei*.

INTRODUCTION

The increase in the price of fishmeal and its growing demand by different food industries has prompted more research on using vegetable proteins in aquaculture diets. One of the vegetable sources considered as an alternative refers to legume grains. However, these present some factors that limit their inclusion, such as being insufficient in both their protein content and digestibility, in addition to having high levels of carbohydrates (polysaccharides with and without starch) and anti-nutritional compounds. Many of these nutritional deficiencies (e.g., limiting levels of sulfur amino acids and tryptophan) can be overcome by the addition of low-cost synthetic amino acids and enzymes or through relevant forms of processing.^{1,2}

Extrusion is a cost-effective processing method that is now widely used to improve the nutritional value of legumes, primarily as a way of reducing the levels of heat-labile, non-nutritional compounds.³ This method, using a combination of moisture, pressure, temperature, and mechanical shear, results in physical and chemical changes, such as ingredient particle size reduction, starch gelatinization, and inactivation of enzymes.⁴ In addition, extrusion enhances widely protein digestibility of plant ingredients.^{5,6}

The use of grain legumes in aquaculture diets has great potential and was used successfully in many experimental aquaculture diets.¹ Soybean products have mostly been used as protein sources in feed for shrimp.⁷⁻¹⁰ Legumes

such as peas,^{11,12} lupin,⁸ canola,¹³ and others (cowpea, green mungbean, and rice bean)¹⁰ have been included in shrimp feeds.² However, information on using extruded chickpea meals in shrimp diets is scarce. Recently, Muñoz-Peñuela et al.¹⁴ found that a 30% substitution of fishmeal with extruded chickpea meal is an adequate dietary inclusion level for tilapia.

The objective of the present work is to evaluate the effect of the dietary substitution of fishmeal with extruded cull chickpea meal on production parameters in the white leg shrimp (*Litopenaeus vannamei*, Boone) experimental production.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Experimental shrimp (2.38 ± 0.15 g) were collected from a growing pond of the semi-intensive farm Acuicola Camaronera Styl, located in Ejido La Culebra (Sinaloa, Mexico).

EXTRUDED CHICKPEA MEAL

Ten kilograms of low-quality chickpeas (variety “Blanco Sinaloa 92”) were ground using a domestic grinder (Grindmaster Model 500, Grindmaster-Cecilware Corp., Louisville, KY, USA), sieved, and conditioned at 23% humidity. Then, the meal was macerated and sieved again at 250 μ m diameter. After that, the chickpea meal was extruded (19-mm-

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diameter single-screw, 127 °C, and 151 rpm screw speed) Brabender 20DN Model No. 8325, C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA), cooled and dried for 24 h to finally be macerated and sieved again (250 µm).

EXPERIMENTAL DIETS

The experimental diets substituted fish meal with extruded chickpea meal at 0, 15, 30, 45, and 60%, for formulating five isoproteic (40%) and isolipidic (11%) diets with semi-purified ingredients (Table 1).

The feed ingredients were processed using various equipment and procedures. Feedstuffs were ground using a Grindmaster Model 505 and then sieved through a 250 µm sieve from FIIC, SA de CV, MX. The ingredients were mixed for 30 minutes in a KitchenAid Model 600 domestic blender (Benson Harbor, MI, USA) and gradually supplemented with fish oil and soy lecithin. Water was added to form a dense dough, which was then pressure-pelleted through a Tororey® meat grinder (Monterrey, NL, MX). The pellets were steamed for 5 minutes and then dried in a draft oven at 60 °C until they contained approximately 10% moisture. The final step involved breaking the diets into 3- to 4-mm sizes, packaging them in plastic bags, and storing them at 5°C until use.

CHEMICAL COMPOSITION

The proximate composition of the experimental diets was determined with three replicates (Table 1). Crude protein and crude lipid were determined using the Kjeldahl method¹⁵ and anhydrous ether in a Soxtec,¹⁶ respectively. The ash content was determined using a muffle furnace.¹⁷ Meanwhile, the crude fiber content was obtained with the phenol-sulfuric acid method.¹⁸ The nitrogen-free extract was obtained by difference. The amino acid content of tested diets (Table 2) was determined based on Tacon et al., 1984.¹⁹

LABORATORY TRIAL

Juvenile shrimp with an average weight of 2.38 ± 0.09 g were randomly selected and stocked at a density of 47 juveniles m⁻² in 60-L plastic tanks (10 organism tank⁻¹) connected to a recirculating system in an indoor laboratory at IPN-CIIDIR-Sinaloa. The diets were tested in triplicate by randomly assigning them to the tanks. After acclimation, 50% of the total water volume of the tanks was replaced daily. The water temperature was maintained at $28.0 \pm 1^\circ\text{C}$ using an aquarium heater (100-W) in the water inlet container. Each tank received constant aeration from a diffuser stone connected to a 2-Hp blower. The dissolved oxygen level was 4.0 ± 0.5 mg L⁻¹, and the pH ranged from 7.66 to 7.98 during the experiment. The mean salinity was 35.0 ± 0.5 psu. Temperature, dissolved oxygen, and salinity were monitored daily using 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. A photoperiod of 12 L: 12 D was maintained using artificial light. Shrimp were fed twice

daily (09.00 and 17.00 h) to apparent satiation, and feces and food waste were removed daily. Shrimp were fed the experimental and control diets for one week before starting the experiment, lasting for 75 days.

The weight of the shrimp was measured every two weeks. Before weighing, the shrimp were placed on absorbent paper to remove excess water. The weight gain (WG) was calculated as $\text{WG (g)} = \text{average final weight} - \text{average initial weight}$. The specific growth rate (SGR) was calculated using the formula proposed by Hopkins²¹: $\text{SGR \%}/\text{day} = 100 \times (\ln \text{wf} - \ln \text{wi})/t$, where wf and wi are the final and initial weight of the shrimp, respectively, and t is the time (days) used for the growth trial. The feed conversion ratio (FCR) was calculated as $\text{FCR} = \text{weight of feed fed (g)}/\text{live weight gain (g)}$. The survival rate (S) was calculated as $\text{S (\%)} = (\text{final number of shrimp per treatment}/\text{initial number of shrimps per treatment}) \times 100$.

For statistical analysis, the data were checked for normality using Lillifors' test and for heteroscedasticity using Bartlett's test. Analysis of variance and Tukey's test were used to compare the final weights, and arcsine transformations were applied to percentages before analysis. STATISTICA package version 7.0 was used for statistical analysis, and the significance level was set at $P < 0.05$ for all analyses. Authors are required to ensure the following: (a) experiments are reproducible, (b) quantitative results derive from at least three replicates, and (c) differences between replicates are not due to random variation.

RESULTS

The results suggest that fish meal can be substituted by extruded chickpea meals at any experimental inclusion level without negative effects on shrimp performance. With the exception of isoleucine at the inclusion levels of 15, 20, and 30%, the amino acid content of the experimental diets was higher than with other 40% crude protein diets²²⁻²⁴ (Table 2). No significant differences ($P > 0.05$) were detected in the final weight, weight gain, and SGR among the treatments, which varied from 11.22 ± 0.9 to 12.42 ± 0.42 g, 8.87 ± 0.5 to 10.11 ± 0.44 g, and 2.08 ± 0.08 to 2.25 ± 0.09 %/d, respectively. No significant differences ($P > 0.05$) were found in FCR and survival among the diets, averaging 2.22 ± 0.28 and $79.92 \pm 4.9\%$, respectively (Table 3).

DISCUSSION

The results show that extruded chickpea meal can partially substitute fish meal as the protein source in diets for *L. vannamei*, representing a viable ingredient with balanced essential nutrient profiles (amino acids). The growth observed in our study was typical of shrimp offered a high-quality practical diet under research conditions.^{10,25,26} Also, high survival in all treatments suggests that the diets were suitable for shrimp growth. The palatability of the diets was acceptable since shrimp consumed the food immediately after being added to the tank. Samocha et al.¹⁰ reported similar growth and survival of *L. vannamei* when

Table 1. Ingredient composition (%) and proximate analysis (%; dry weight) of five experimental diets for juvenile *Litopenaeus vannamei* (mean \pm SD, n=3)*.

Ingredients	0%	15%	30%	45%	60%
Fish meal	330	280.5	231	181.5	132
Extruded chickpea	0	150	280	415	546.9
Soy paste	200	200	200	200	200
Cellulose	348.9	248.4	167.9	82.4	0
Binder (grenetine)	40	40	40	40	40
Fish oil	40	40	40	40	40
Soybean lecithin	40	40	40	40	40
Vitamins premix	0.1	0.1	0.1	0.1	0.1
Mineral premix	1.0	1.0	1.0	1.0	1.0
Moisture (%)	9.86 \pm 0.11	8.15 \pm 0.10	7.32 \pm 0.39	9.29 \pm 0.55	10.06 \pm 0.17
Protein (%)	39.70 \pm 0.44	39.18 \pm 0.74	40.12 \pm 0.02	40.22 \pm 0.06	39.24 \pm 0.21
Lipid (%)	10.06 \pm 0.06	10.07 \pm 0.60	11.40 \pm 0.09	11.39 \pm 0.04	11.62 \pm 0.11
Ash (%)	6.15 \pm 0.05	5.92 \pm 0.03	5.82 \pm 0.04	5.19 \pm 0.14	4.60 \pm 0.02
Fiber (%)	19.92 \pm 0.12	15.67 \pm 0.33	9.25 \pm 0.05	4.54 \pm 0.18	0.18 \pm 0.03
NFE*	24.17	29.16	33.41	38.66	44.36

Table 2. Amino acid composition (g/kg, dry matter) of experimental diets compared with a shrimp diet (40% crude protein).

Amino acid	Substitution (%)					Recommended dietary level
	0	15	30	45	60	
Arginine	3.63	3.99	4.29	4.59	4.89	2.17 ³
Cysteine	0.67	0.74	0.79	0.85	0.91	0.38 ³
Methionine	1.14	1.14	1.12	1.10	1.08	0.76 ²
Threonine	2.38	2.46	2.52	2.59	2.65	1.09 ³
Isoleucine	0.19	0.25	0.30	0.36	0.41	0.38 ³
Leucine	3.47	3.66	3.80	3.95	4.10	1.08 ³
Lysine	1.14	1.22	1.27	1.33	1.38	0.62 ³
Valine	1.60	1.77	1.90	2.03	2.16	0.95 ¹
Tyrosine	2.89	3.14	3.32	3.53	3.72	1.96 ¹
Tryptophan	3.15	3.29	3.37	3.47	3.56	2.06 ³
Phenylalanine	1.67	1.78	1.86	1.94	2.02	1.34 ¹
Histidine	1.91	2.07	2.18	2.31	2.43	1.19 ³

²⁰ Tacon et al. (1999).¹⁷ Fox et al. (2010).¹⁵ Nunes et al. (2014).

totally replacing fish meal with a mixture of extruded soybean and poultry by-product meal supplemented with egg.

The overall results suggest that fish meal can be partially substituted with the tested ingredients without negatively affecting shrimp performance. The SGR was similar to the 1.9 reported by Davis and Arnold⁹ feeding *L. vannamei* with co-extruded soybean/poultry by-product meal during 42 days, but lower than the 3.9 reported by Suárez et al.²⁷ when the fish meal was replaced with soy and canola meals. We observed a favorable response of shrimp to the 60% substitution diet, possibly due to its amino acid content. On the contrary, after 50% fish meal substitution by com-

mercial vegetable sources, Sánchez-Muro et al.²⁸ reported a decrease in shrimp growth. Differences in results could be partially explained by the vegetable sources, inclusion level, diet processing, and shrimp age, among others. In this study, the extrusion technique reduces antinutritional factors and decreases trypsin inhibitors contained in the chickpea meal, increasing diet digestibility.²⁹ However, the obtained survival (>77.7 %) could be related to the heat-stable anti-nutritional ingredients contained in the experimental meals. Vikas et al.³⁰ reported that survival was reduced by the presence of saponins, phytic acid and glucosinolates (heat-stable), in the plant feedstuffs.

Table 3. Mean final weight, weight gain, and survival of juvenile *L. vannamei* fed different diets based on extruded cull chickpea under laboratory conditions (mean \pm SD; n = 3).

Diet	Initial mean weight (g)	Final mean weight (g)	Weight gain (g)	SGR (%/g)	FCR	Survival (%)
0%	2.35 \pm 0.08 ^a	11.22 \pm 0.47 ^a	8.87 \pm 0.51 ^a	2.08 \pm 0.08 ^a	2.14 \pm 0.41 ^a	88.8 \pm 9.6 ^a
15%	2.45 \pm 0.15 ^a	11.86 \pm 1.12 ^a	9.41 \pm 1.03 ^a	2.10 \pm 0.10 ^a	2.10 \pm 0.31 ^a	77.7 \pm 9.6 ^a
30%	2.52 \pm 0.15 ^a	12.29 \pm 1.06 ^a	9.77 \pm 0.93 ^a	2.11 \pm 0.05 ^a	2.25 \pm 0.23 ^a	77.7 \pm 9.6 ^a
45%	2.31 \pm 0.11 ^a	11.38 \pm 0.10 ^a	9.07 \pm 0.10 ^a	2.13 \pm 0.05 ^a	2.22 \pm 0.16 ^a	77.7 \pm 9.6 ^a
60%	2.31 \pm 0.14 ^a	12.42 \pm 0.42 ^a	10.11 \pm 0.44 ^a	2.25 \pm 0.09 ^a	2.23 \pm 0.31 ^a	77.7 \pm 9.6 ^a

Values in the same column with different superscripts are significantly different ($P < 0.05$)

Some studies suggest that fish meal can be totally substituted with alternative ingredients in diets for *L. vannamei*.^{20,31} In contrast to reports by those authors, our diets did not impair shrimp growth at any substitution level tested; in fact, it significantly improved it up to 60% substitution. So far, the information indicating that the growth of *L. vannamei* can be significantly improved when substituting fish meal with plant ingredients at high levels is null. According to our results, 13% inclusion of fish meal was optimal for shrimp growth under laboratory conditions, closely approximating the low fish meal inclusion levels of 7.5 to 12.5% reported by Fox et al.³² without compromising shrimp performance.

It is well known that the presence of anti-nutritional factors in plant protein impairs the growth of aquaculture species.³³ One of the best strategies to overcome this limitation includes replacing fish meal with extruded plant protein, which can reduce the anti-nutritional factors.³⁴ In conclusion, extruded chickpea meal can be considered a potential alternative ingredient for substituting fish meal in practical diets of *L. vannamei*. To evaluate its possible economic benefit, it is recommended to conduct trials including extruded cull chickpea meal in shrimp diets at commercial farms.

ACKNOWLEDGMENTS

The authors thank to Instituto Politécnico Nacional (SIP-IPN 20120542; 20131517; 20141467), COFAA, and EDI for the financial and logistic support. Tejeda-Miramontes, J. P. is a recipient of a Graduate studies fellowship from CONACYT and Instituto Politécnico Nacional (BEIFI Grant).

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Submitted: May 24, 2023 CST. Accepted: July 27, 2023 CST.

Published: August 31, 2023 CST.



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