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Nutritional value of cowpea (*Vigna unguiculata* L. Walp) meals as ingredients in diets for Pacific white shrimp (*Litopenaeus vannamei* Boone)

M.E. Rivas-Vega [a], E. Goytortúa-Bores [b], J.M. Ezquerra-Brauer [c], M.G. Salazar-García [b], L.E. Cruz-Suárez [c], H. Nolasco [a], R. Civera-Cerecedo [a,*]

[a] *Laboratorio de Nutrición Acuícola, Centro de Investigaciones Biológicas del Noroeste, (CIBNOR), Mar Bermejo No. 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, México*
[b] *Universidad de Sonora, Rosales y Transversal s/n, Hermosillo, Sonora 83000, México*
[c] *Universidad Autónoma de Nuevo León, Ciudad Universitaria A.P. F-56, San Nicolás de los Garza, Nuevo León 66450, México*

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Abstract

The nutritional value of cowpea (*Vigna unguiculata* L. Walp) meals, as ingredients in diets for *Litopenaeus vannamei*, was evaluated. Five experimental meals were prepared in the laboratory: whole raw cowpea (WRC), dehulled (DC), cooked (CC), germinated (GC) and extruded (EXC). The crude protein content of WRC (26.1%) increased after germination (29.5%). Carbohydrates ranged from 69.4% to 85.9%. The trypsin inhibitor activity of WRC meal was low (7.52 U/mg dry matter), and was reduced or eliminated by cooking and extrusion. Apparent digestibility of dry matter, protein and carbohydrate of the diet containing whole raw cowpea meal (71.1%, 85.9% and 76.7%, respectively) was similar to the control diet. Cooking and extruding of cowpea significantly increased dry matter, protein and carbohydrate digestibility in the diets. The results suggest that cowpea meals are good sources of nutrients and can be used as ingredients in diets for *L. vannamei*.

Keywords: Cowpea meals; *Vigna unguiculata*; Feed ingredients; Digestibility; *Litopenaeus vannamei*

1. Introduction

Growth of the aquaculture industry has increased fish meal demand, causing uncertainty in its availability (Davis et al., 2004). Balanced rations should preferably include conventional and novel ingredients available to each locality (New, 1987). Investigations to evaluate the use of ingredients of vegetal origin to replace traditional ingredients in aquaculture feeds, such as fish and soybean meals, have been conducted (Kikuchi, 1999; Tacon, Webster, & Martinez, 1984). However, due to the presence of antinutritional factors, their use has not provided a desirable level of fish growth (Brunson, Romaire, & Reigh, 1997; Stickney et al., 1996). An alternative to eliminate or reduce the presence of antinutritional factors in plant origin ingredients is the use of appropriate processes, such as dehulling and extruding, which enhance the nutritional quality of the ingredient (Cruz-Suárez, Ricque-Marie, Tapia-Salazar, McCallum, & Hickling, 2001).

Cowpea (*Vigna unguiculata* L. Walp) is a widely distributed leguminous plant that is used as a feed ingredient in diets for poultry and pigs (Murillo-Amador, Troyo-Diéguez, García-Hernandez, Landa-Hernández, & Larrinaga-Mayoral, 2000). Its world production reached 3722 thousand metric tonnes in 2003 (FAO, 2004), and

* Corresponding author. Tel.: +52 612 123 8407; fax: +52 612 125 3625.
E-mail address: rcivera04@cibnor.mx (R. Civera-Cerecedo).

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it is a highly digestible ingredient in diets for aquatic organisms such as *Penaeus monodon* (Eusebio, 1991) and tilapia (Keembiyehetty & de Silva, 1993; Olvera-Novoa et al., 1997). However, the nutritional value of cowpea has not been evaluated for Pacific white shrimp, *Litopenaeus vannamei*, species of high commercial interest in aquaculture. In addition, *L. vannamei* is, among penaeid species, probably the most suited for utilizing nutrients from vegetal origin, due to its dietary habits (Cuzon, Brito, Jiménez-Yan, Brito, & Gaxiola, 2004).

The objective of the present study was to obtain cowpea (*Vigna unguiculata*) meals by different processes: dehulling, cooking, germination and extrusion, and to evaluate their nutritional value as ingredients in balanced diets for juveniles of *L. vannamei*.

2. Materials and methods

2.1. Materials

Cowpea beans (*Vigna unguiculata* L. Walp) were obtained from Sierra de Álamos, Sonora, Mexico. To determine their nutritional value as an ingredient in diets for shrimp, raw whole cowpeas (RWC) were submitted to different processes to give: (1) Dehulled (DC) in a Strong-Scott 17810 dehulling machine; (2) Cooked (CC); beans were soaked in distilled water (1:10 cowpea: water (w/v)) during 105 min at room temperature, boiled for 20 min (Anduaga-Cota, Cota Gastélum, Falcón-Villa, Yánez-Farías, & Barrón-Hoyos, 2002), and dried in a convection oven at 40 °C for 24 h; (3) Germinated (GC) on humid filter paper in a Biotronette Mark III, Lab-Line germination chamber at 33 °C and 50% relative humidity for 3 d in complete darkness, then dried in a convection oven at 40 °C for 24 h; (4) Extruded (EXC) in a single screw Brabender, Do-Corder extruder with a temperature of entrance of 80 °C and of exit of 180 °C, using 10–12 bars of pressure. The different cowpea products obtained were milled in a pulveriser PULVEX 200, sifted through a 250 μm mesh sieve, and stored at 4 °C until used.

2.2. Formulation and elaboration of diets

A control diet containing 34% protein, 7% lipids, and 1% Cr_2O_3, used as indirect marker for in vivo digestibility determinations, was formulated using the software MIXIT-WIN. Five experimental diets containing 84% of the control diet, 15% of the test meals, and 1% Cr_2O_3 were also formulated (Table 1). Prior to preparing the experimental diets, all ingredients were pulverized and sieved through a 250 μm mesh sieve. The dry ingredients of each diet were mixed thoroughly in a food mixer before an oil mix (fish oil and soybean lecithin) was added. After the oil was dispersed, water was added (approximately 40% of the total "as is" ingredient weight) and mixed. The resulting mixture was pressure-pelleted using a meat grinder and a 2 mm die. The pellets were dried in a forced-air oven at 45 °C for 12 h.

2.3. Chemical analysis

Moisture, crude protein, ash, lipid and crude fibre were determined according to AOAC (1990) methods. The nitrogen-free extract was calculated by difference; the digestible carbohydrates were determined using the anthrone method (Dreywood, 1946). To determine calcium and phosphorus contents, 0.3 g samples were digested in nitric acid 70 (%) at 170 °C for 10 min, using a CEM Mars 5× oven. Calcium was determined by reading the digested samples in an atomic absorption GBC Avanta PM spectrophotometer (Sapp & Davidson, 1991). Phosphorus was determined using the yellow colour of the vanadomolibdophosphoric complex in a system acidified with nitric acid, and the reading was done using micro-plaque Labsystems, Multiscan Ascent (Jackson, 1958).

Chromic oxide was quantified by the method described by Olvera-Novoa (1994). Fifty mg sample were digested in 5 ml of nitric acid, and later in perchloric acid at 300 °C until a red ring in the surface of the solution appeared. After digestion, 25 ml of distilled water were added. Absorbance was read at 350 nm.

2.4. Trypsin inhibitor activity

Trypsin inhibitor activity was determined following the methodology described by Kakade, Rackis, McGhee, and Poski (1974), using BAPNA as substrate (0.92 mM in buffer 0.05M Tris/0.02 M $CaCl_2$, pH 8.2). One gram/ml of finely ground sample was extracted in 50 ml of 0.01 N NaOH for 3 h; the pH was maintained within the range 8.5 to 9.0. Two ml of the extract and 2 ml of trypsin solution 0.002% (Type I, of bovine pancreas, SIGMA Cat. # T 8003) in 0.001 M HCl were mixed; the reaction began when adding 5 ml of substrate at 37 °C. After 10 min, the reaction was stopped by the addition of 1 ml of 30% acetic acid. The reaction mixture was filtered through filter paper (Whatman No. 3) and the absorbance read at 410 nm. The activity was interpreted as the increment of 0.01 units of absorbance at 410 nm for 10ml of reaction mixture. Results are expressed in terms of trypsin units inhibited (TUI) by one mg of dry sample (TUI/mg sample) and TUI/mg protein.

2.5. In vitro digestibility

Digestibility of cowpea meals was determined in vitro, following the method of AOAC (1990). One

Table 1
Ingredient composition of the diets (g/100 g diet) for juvenile, *L. vannamei* used to determine the in vivo digestibility of the different cowpea meals[a]

Ingredient	Control diet	WRCD	DCD	CCD	GCD	EXCD
Whole raw cowpea (WRC)[a]	0.00	15.00	0.00	0.00	0.00	0.00
Dehulled (DC)[a]	0.00	0.00	15.00	0.00	0.00	0.00
Cooked (CC)[a]	0.00	0.00	0.00	15.00	0.00	0.00
Germinated (GC)[a]	0.00	0.00	0.00	0.00	15.00	0.00
Extruded (EXC)[a]	0.00	0.00	0.00	0.00	0.00	15.00
Wheat flour[b]	35.93	30.49	30.49	30.49	30.49	30.49
Soybean meal[b]	25.00	21.21	21.21	21.21	21.21	21.21
Fish meal (sardine)[b]	20.00	16.97	16.97	16.97	16.97	16.97
Kelp meal[b]	4.00	3.39	3.39	3.39	3.39	3.39
Corn gluten [c]	3.77	3.20	3.20	3.20	3.20	3.20
Cod liver oil [d]	3.00	2.55	2.55	2.55	2.55	2.55
Soy lecitin[e]	3.00	2.55	2.55	2.55	2.55	2.55
Vitamin premix[f]	1.80	1.53	1.53	1.53	1.53	1.53
Dibasic sodium phosphate[g]	1.20	1.02	1.02	1.02	1.02	1.02
Cholesterol[h]	0.50	0.42	0.42	0.42	0.42	0.42
Mineral premix[i]	0.50	0.42	0.42	0.42	0.42	0.42
Choline chloride 62%[b]	0.20	0.17	0.17	0.17	0.17	0.17
Vitamin C[j]	0.090	0.076	0.076	0.076	0.076	0.076
BHT[k]	0.004	0.0034	0.0034	0.0034	0.0034	0.0034
Chromic oxide[l]	1.00	1.00	1.00	1.00	1.00	1.00

[a] Prepared in the laboratory from cowpea *Vigna unguiculata*, Sierra de Alamos, Sonora, México.
[b] Promotora Industrial Acuasistemas, S.A. de C. V., La Paz, BCS, México.
[c] Gluten y almidones industriales S.A. de C. V., México, D.F.
[d] Farmacia Paris, S.A. de C.V., México, D.F.
[e] ODONAJI. Distribuidora de Alimentos Naturales y Nutricionales S.A. de C.V., México, D.F.
[f] Composition of the vitamin premix (g/kg premix): Vit. A 5, D_3 0.001, E 8, Menadione 2, B_1 0.5, B_2 3, B_6 1, DL-Ca-Pantothenate 5, nicotinic acid 5, H 0.05 inositol 5, B_{12} 0.002, folic acid 0.18, α-cellulose 865.266.
[g] SIGMA Cat No. S-0876. SIGMA–ALDRICH Chemical Company, St. Louis, MO, USA.
[h] SIGMA Cat. No. C-8503. SIGMA–ALDRICH Chemical Company, St. Louis, MO, USA.
[i] Composition of the mineral premix (g/100 g premix): $CoCl_2$ 0.004, $CuSO_4 \cdot 5H_2O$ 0.25, $FeSO_4 \cdot 7H_2O$ 4, $MgS0_4 \cdot 7H_2O$ 28.398, $MnS0_4 \cdot H_2O$ 0.65, KI 0.067, Na_2SeO_3 0.01, $ZnSO_4 \cdot 7H_2O$ 13.193, α-cellulose 53.428.
[j] Stay C 35% active agent. Roche, México, D.F.
[k] Butylated hydroxytoluene, ICN Cat. No. 101162. Aurora, OH, USA.
[l] Aldrich Cat No. 20,216-9. SIGMA–ALDRICH Chemical Company, St. Louis, MO, USA.

gram/ml of sample was incubated in a solution of 0.002% pepsin (from porcine gastric mucosa, SIGMA # Cat. P-7000) in 0.075 N HCl for 16 h at 45 °C. The reaction solution was filtered to retain the insoluble fraction. Protein content was determined using the Kjeldahl method; a blank was used without pepsin in all determinations. The digestibility was calculated using the following formula: % in vitro digestibility = (% residual protein without pepsin − % of residual protein with pepsin)/(% residual protein without pepsin).

2.6. In vivo digestibility

Juvenile white shrimp *L. vannamei* with a mean weight of 15.4 ± 0.9 g were used. Shrimps were stocked in 60 l rectangular tanks (58 × 48 × 25 cm) at a density of 5 shrimp/tank. Three replicate tanks were randomly assigned for each diet. Shrimps were maintained in filtered seawater at 27.1 ± 0.005 °C, 39.7 ± 0.004 ups, and 4.4 ± 0.007 mg/ml dissolved oxygen. Shrimps were fed ad libitum three times daily during 7 d before beginning the feces collection. One hour after each feeding faecal strands were syphoned out gently, using a Pasteur pipette and then they were gently rinsed with distilled water and frozen at −80 °C until analysed. At the end of the trial, collected feces from each tank were pooled and freeze-dried. The diets and fecal samples were analyzed for chromic oxide (Cr_2O_3), crude protein, carbohydrates, phosphorus and calcium. The apparent digestibility coefficients (ADC) for dry matter, protein, carbohydrate, phosphorus and calcium were determined using the following formulas (Maynard, Loosli, Hintz, & Warner, 1981): ADC of dry matter (%) = 100 − [(%Cr_2O_3 in feed/%Cr_2O_3 in feces) × 100]. ADC of nutrients (%) = 100 − 100[(%Cr_2O_3 in feed/%Cr_2O_3 in feces) × (% nutrient in feces/% nutrient in feed)].

2.7. Enzymatic activity in the hepatopancreas of *L. vannamei*

2.7.1. General

At the end of the digestibility trial, the hepatopancreas tissues from 6 shrimps in molting stage C/dietary treatments were extracted, and frozen to −20 °C until

analysed. Samples from each treatment were homogenized in three volumes of distilled water. The homogenate was centrifuged at 11,300*g* at 4 °C for 20 min. The supernatant was used for the following determinations.

2.7.2. Total alkaline proteases

Azocasein was used as substrate in 1% Tris–HCl Buffer, 50 mM, pH 7.5; 0.5 ml of the buffer was mixed with 0.5 ml of azocasein to 1%, running a blank at the same time. The reaction began when adding 10 μl of the extract and was stopped after 10 min by adding 0.5 ml of 20% trichloroacetic acid. To the blank was added trichloroacetic acid before the substrate. It was centrifuged at 16,500 *g* for 5 min at 4 °C and the absorbance was read at 440 nm. The activity is reported as (Abs440/min)/mg protein (Erlanger, Kokowsky, & Cohen, 1961).

2.7.3. Trypsin activity

Benzoyl-Arg-p-nitroaniline (BAPNA) was used as substrate. It was dissolved in 1 ml of DMSO, and made up to 100 ml with Tris buffer (50 mM, pH 7.5), containing 20 mM $CaCl_2$. Ten microlitres of the enzymatic extract were added to 1.25 ml of the substrate solution; after 10 min the reaction was stopped with 0.25 ml of acetic acid 30%, and the absorbance was read at 410 nm. The trypsin-like activity is reported as activity units (Abs410/min)/mg protein (Erlanger et al., 1961).

2.7.4. Chymotrypsin activity

The chymotrypsin-like activity was measured using succinyl-$(Ala)_2$-Pro-Phe-p-nitroanilide (SAPNA) as substrates. Twenty microlitres of the enzymatic preparation were added to a solution containing 50 μl substrate (0.02 mm SAPNA) and 590 μl of Tris–HCl buffer (pH 7.8) containing 0.01 M $CaCl_2$. The reaction was stopped after 5 min by adding 0.25 ml of 30% acetic acid. Absorbance was read to 410 nm. One unit of chymotrypsin-like activity is reported as activity units (Abs410/min)/mg protein (García-Carreño, Hernandez-Cortes, & Haard, 1994).

2.7.5. Amylase activity

Corn starch was used as substrate. Five μl of the enzymatic extract, 500 μl, Tris–HCl buffer, 50 mM, pH 7.5, and 500 μl of soluble starch 1% were mixed. After 10 min of incubation at 37 °C, 200 μl of 2N sodium carbonate, and 1.5 ml of DNS were added, the mixture was agitated and warmed to a boil in a water bath for 15 min; later 7.3 ml of distilled water were added. The absorbance was read at 550 nm, and one blank was run at the same time. The amylase units are reported as Units(Abs 550/min)/mg protein (Vega-Villasante, Nolasco, & Civera, 1993).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant differences among treatments, with a confidence level of $p = 0.05$. When significant differences existed, Tukey's multiple comparison of means test was used. Procedures available in STATISTICA™ 7.0 (StatSoft, Inc., Tulsa, OK, USA): were used to conduct the statistical tests.

3. Results and discussion

3.1. Chemical composition

The proximate composition, carbohydrates, calcium and phosphorus contents of cowpea meals are shown in Table 2. Crude protein content of whole raw cowpea (26.14%) is within the range of values reported for other varieties of cowpea (Kochhar, Walker, & Pike, 1988; Oluwatosin, 1997; Tshovhote, Nesamvuni, Raphulu, & Gous, 2003). The increase in crude protein content, after germination, can be attributed to production of growth enzymes (Sunday, Monday, & Juliet, 2001). Lipid content in all cowpea meals was low, ranging from 1.64% to 0.80%. Lipid content was decreased by the effect of dehulling, and increased by cooking and germination, but was not affected by

Table 2
Proximate composition, carbohydrates, calcium and phosphorus contents of cowpea (*Vigna unguiculata*) meals

Ingredient	Moisture (%)	Crude protein (%)	Lipids (%)	Crude fibre (%)	Ash (%)	NFE[A] (%)	Carbohydrates (%)	Calcium (mg/l00g)	Phosphorus (mg/l00 g)
Whole raw cowpea	7.92 ± 0.96^{c}	26.1 ± 0.27^{ab}	1.05 ± 0.01^{b}	2.60 ± 0.20^{d}	3.93 ± 0.14^{b}	66.28	74.8 ± 2.81^{ab}	34.7 ± 0.7^{b}	30.9 ± 2.0^{ab}
Dehulled	7.85 ± 0.04^{c}	25.6 ± 0.10^{a}	0.80 ± 0.01^{a}	1.29 ± 0.04^{b}	3.75 ± 0.07^{b}	68.61	78.9 ± 3.50^{ab}	37.6 ± 0.6^{b}	17.0 ± 02^{a}
Cooked	6.98 ± 0.11^{b}	27.2 ± 0.15^{b}	1.64 ± 0.10^{d}	1.92 ± 0.13^{c}	2.85 ± 0.02^{a}	66.3	81.2 ± 7.02^{b}	29.5 ± 0.5^{a}	38.5 ± 0.8^{ab}
Germinated	6.36 ± 0.04^{a}	29.5 ± 1.17^{c}	1.40 ± 0.04^{c}	2.12 ± 0.01^{c}	4.23 ± 0.02^{c}	62.74	85.9 ± 3.70^{b}	29.2 ± 0.2^{a}	50.0 ± 3.9^{b}
Extruded	6.83 ± 0.04^{b}	25.6 ± 0.20^{a}	0.96 ± 0.02^{b}	0.70 ± 0.09^{a}	10.59 ± 0.04	62.19	69.4 ± 1.78^{a}	50.0 ± 2.0^{b}	578 ± 10.0^{c}

Values are means of three replicates (g/100 g dry matter, except moisture) ±SD.
Values within the same column with different superscripts are significantly different ($p < 0.05$).
[A] Nitrogen-free extract.

extrusion, probably due to the low content of lipids (Cheng & Hardy, 2003).

The cowpea meals prepared were low in crude fibre content, compared to the whole grain (4.42-6.34%), reported for different varieties (Eusebio, 1991; Tacon, 1989; Tshovhote et al., 2003). It was observed that all the processes significantly reduced Fibre content compared to whole raw cowpea. In contrast, carbohydrate content was not affected by processing.

Ash content decreased in the cooked meal, probably due to loss of minerals (e.g., calcium) during soaking and boiling in water, even though no effect on phosphorus content was observed. Germination, and particularly extrusion, significantly increased the ash and phosphorus contents. During the extrusion process, high temperatures were attained (180 °C) which may have mineralized some of the organic material in the seeds. More studies are needed to optimize the extrusion conditions of cowpea and to avoid organic matter loss during the process.

3.2. Trypsin inhibitor activity

Table 3 shows the trypsin inhibitor activity (TIA) in cowpea meals. The TIA in the raw whole cowpea meal (31.6 TIU/mg protein) is slightly higher than that reported by Ologhobo and Fetuga (1984) for an other variety of cowpea (23.7 TIU/mg protein), but lower than values reported for other beans, such as *Phaseolus vulgaris* (23.1 and 10.9 TIU/mg of sample) (Dhurandhar & Chang, 1990) and soybean (*Glycine max*) 106 TIU/mg of sample (Kakade et al., 1974).

A significant decrease in TIA was observed after cooking for 20 min, although Ologhobo and Fetuga (1984) report that, after 15 min of cooking, the TIA in cowpea disappears. The differences in the results can be attributed to the different cooking methods used. In the case of the extruded cowpea meal, the trypsin inhibitor activity was eliminated, probably due to its complete inactivation by the high pressure and temperature used during this process. This effect is also observed in the experimental diets, since the diet containing extruded cowpea meal had the lowest TIA content (Table 4).

3.3. Digestibility

The in vitro digestibility in the thermally treated meals (cooked and extruded) was significantly higher than of whole raw cowpea (Table 3). Dehulled meal also presented high digestibility with pepsin. Leguminous plants constitute good energy and protein sources; however, the suitability of these nutrients for aquatic organisms is not well known (Davis et al., 2004). One of the disadvantages of the use of vegetable ingredients in animal feeds is that they contain antinutritional factors such as protease inhibitors, phytic acid, and tannins, that cause that the nutrients to be less available for the organisms. An alternative, to diminish these effects, is the use of vegetal inputs to processes that allow the destruction or inactivation of the antinutritional factors, as has been reported (Cruz-Suárez et al., 2001) and observed here.

The in vivo apparent digestibility (AD) of dry matter, crude protein and carbohydrates of the control diet, and the experimental diets containing 15% of the different cowpea meals, is shown in Table 5. Dry matter AD of the diets varied from 68.1% to 74.2%.

The inclusion of whole raw cowpea meal in the diet did not negatively affect dry matter, protein or carbohydrate digestibility. These results are in agreement with those reported for *Penaeus monodon* (Eusebio, 1991) and tilapia (Keembiyehetty & de Silva, 1993; Olvera-Novoa et al., 1997), where the cowpea has been reported as a highly digestible ingredient in diets for aquatic organisms. Nevertheless, it must be noticed that cowpea, like other leguminous seeds, has a low methionine content (Kochhar et al., 1988). This is an essential amino acid for animals; hence its use as a protein source in shrimp feeds is compromised, unless the feed is enriched with pure amino acids or combined with other protein sources. Further research is needed to determine the optimal inclusion level of cowpea meals in the diet, and to asses their effects on shrimp survival and growth.

Compared to the control diet extruded, cooked, and germinated meals significantly improved dry matter digestibility of the diet. Cruz-Suárez et al. (2001) reported that the inclusion of extruded pea (*Pisum sativum*) meal in diets for the shrimp, *Litopenaeus*

Table 3
Trypsin inhibitor activities (TIA) and in vitro digestibilities of cowpea (*Vigna unguiculata*) meals obtained by different processes

Ingredient	TIA (TIU[A]/mg dry matter)	TIA (TIU[A]/mg protein)	In vitro digestibility[B]
Whole raw cowpea	7.52 ± 0.34^{c}	31.6 ± 1.46^{cd}	57.2 ± 6.05^{a}
Dehulled	7.70 ± 0.28^{c}	33.4 ± 1.24^{d}	74.2 ± 4.03^{b}
Cooked	2.68 ± 0.14^{b}	10.6 ± 0.56^{b}	88.7 ± 2.07^{c}
Germinated	7.92 ± 0.36^{c}	28.7 ± 1.34^{c}	66.3 ± 2.46^{ab}
Extruded	0.00^{a}	0.00^{a}	85.6 ± 0.20^{c}

Values within the same column with different superscripts are significantly different ($p < 0.05$).
[A] Trypsin units inhibited.
[B] AOAC (1990).

Table 4
Proximate composition[A] (g/100g dry matter, except moisture) and trypsin inhibitor activity (TIA) of the diets used in the digestibility bioassay with *L. vannamei*

DIET	Moisture (%)	Crude protein (%)	Lipids (%)	Crude fibre (%)	Ash (%)	NFE[B] (%)	TIA(TIU[A]/mg dry matter)
Control	6.63 ± 0.05	33.9 ± 0.16	8.47 ± 0.04	1.74 ± 0.21	15.9 ± 0.05	47.4	4.81 ± 0.06[b]
WRCD[C]	5.98 ± 0.02	32.5 ± 0.01	7.15 ± 0.05	1.64 ± 0.36	14.6 ± 0.05	38.2	3.63 ± 0.05[ab]
DCD[D]	7.27 ± 0.11	32.2 ± 0.39	7.07 ± 0.08	1.01 ± 0.03	15.7 ± 0.09	36.7	4.60 ± 0.03[b]
CCD[E]	6.89 ± 0.12	32.8 ± 0.30	7.43 ± 0.03	1.45 ± 0.28	15.2 ± 0.12	36.2	4.33 ± 1.01[b]
GCD[F]	6.30 ± 0.14	33.4 ± 0.42	7.00 ± 0.01	0.94 ± 0.01	14.9 ± 0.15	37.4	3.45 ± 0.45[ab]
EXCD[G]	6.76 ± 0.10	33.5 ± 0.26	6.87 ± 0.10	1.94 ± 0.08	15.3 ± 0.06	35.7	2.31 ± 0.48[a]

[A] Values are means of three replicates ± SD.
[B] Nitrogen-free extract.
[C] Whole raw cowpea diet.
[D] Dehulled cowpea diet.
[E] Cooked cowpea diet.
[F] Germinated cowpea diet.
[G] Extruded cowpea diet.

Table 5
Apparent digestibility coefficients (% ± SD) for dry matter, crude protein, and carbohydrates of the control diet and the experimental diets containing cowpea meals fed to juvenile *L. vannamei*

Diet	Dry matter	Crude protein	Carbohydrates
Control diet	70.2 ± 0.14[a]	85.8 ± 0.40[ab]	77.7 ± 0.56[a]
WRCD[A]	71.1 ± 1.20[ab]	85.9 ± 0.27[ab]	76.7 ± 1.0[a]
DCD[B]	68.1 ± 0.15[a]	84.3 ± 0.28[a]	81.3 ± 1.06[b]
CCD[C]	75.3 ± 0.37[c]	88.3 ± 0.25[c]	82.5 ± 0.83[b]
GCD[D]	75.1 ± 1.12[c]	87.2 ± 1.53[bc]	81.2 ± 0.27[b]
EXCD[E]	74.2 ± 0.88[bc]	87.9 ± 0.44[c]	81.7 ± 0.27[b]

Values are means of three replicates ± SD.
[A] Whole raw cowpea diet.
[B] Dehulled cowpea diet.
[C] Cooked cowpea diet.
[D] Germinated cowpea diet.
[E] Extruded cowpea diet.

stylirostris, significantly increased dry matter digestibility and attributed it to the gelatinization of starches caused by the thermal treatment during extrusion, since this process causes the loss of the molecular order of starch granules and makes them susceptible to enzymatic hydrolysis (Thiessen, Campbell, & Adelizi, 2003). In the present study, the digestibility of cooked cowpea meal diet increased significantly, although the degree of gelatinization of starches was not determined.

Cooked and extruded meals significantly increased crude protein AD in diets that contained these meals. This can be attributed to the thermal treatments causing denaturation of the trypsin inhibitor. Another effect of thermal treatment is the partial denaturation of proteins that makes them more digestible, compared to the native proteins (Fennema, 1996). On the other hand, several studies in penaeid shrimp have reported that there is no significant effect of dehulling on apparent protein digestibility (Cruz-Suárez et al., 2001; Davis et al., 2004; Eusebio, 1991) and our results are in agreement with those studies.

Although no significant correlation between in vitro protein digestibility of the ingredients and in vivo digestibility of the diets that contained them was found, apparent protein digestibilities of diets follow a similar pattern to results of pepsin digestibility of the meals. The apparent carbohydrate digestibility of diets containing dehulled, cooked, germinated and extruded cowpea meals was significantly higher than that of diet containing raw cowpea. Nnanna and Phillips (1990) reported, in rats, that germination of cowpea significantly improved starch digestibility. These authors attributed this phenomenon to the production of amylolytic enzymes during germination, which serves to degrade starch and to use it as an energy source for the development of the plant. They also reported that, during germination of the seeds at 30 °C, the oligosaccharides (3.75% of dry matter) were completely degraded, contributing to the increase in carbohydrate digestibility. In the case of the cooked and extruded cowpea meals of our study, it is expected that the digestibility of carbohydrates increases, since the thermal treatments gelatinize the starches, which allows better digestion.

3.4. Enzymatic activity in the hepatopancreas of white shrimp fed diets containing different cowpea meals

Table 6 shows the digestive enzymatic activity of shrimp fed diets containing cowpea meals. No effect of the dietary treatments on soluble protein, total alkaline protease or amylase activities was found. In contrast, trypsin activity was significantly increased by the inclusion of cowpea meal in the feeds, independently of the treatment. Some reports indicate that the digestive enzymatic activity is modified by effect of the starving, age, or molting stage of shrimp (Lee, Smith, & Lawrence, 1984; Le Moullac et al., 1994; Maugle, Deshimaru, Katayama, & Simpson, 1982), as well as the quantity and quality of protein in the feed (Ezquerra, García-Carreño, & Haard, 1997; Ezquerra, García-Carreño,

Table 6
Soluble protein and specific enzymatic activity in the hepatopancreas of white shrimp (*L. vannamei*) fed diets containing different cowpea (*Vigna unguiculata*) meals

DIET	Soluble protein (mg/ml)	Total protease (U/mg Protein)	Trypsin (U/mg Protein)	Chymotrypsin (U/mg Protein)	Amylase (U/mg Protein)
Control diet	1.42 ± 0.11^{a}	2.11 ± 0.49^{a}	1.90 ± 0.53^{a}	0.251 ± 0.031^{c}	32.3 ± 4.54^{a}
WRCD[A]	1.36 ± 0.05^{a}	1.63 ± 0.39^{a}	2.40 ± 0.14^{b}	0.085 ± 0.013^{ab}	34.9 ± 2.23^{a}
DCD[B]	1.35 ± 0.06^{a}	2.31 ± 0.43^{a}	2.41 ± 0.12^{b}	0.120 ± 0.053^{b}	29.2 ± 5.78^{a}
CCD[C]	1.29 ± 0.06^{a}	2.16 ± 0.26^{a}	2.37 ± 0.19^{b}	0.052 ± 0.015^{a}	29.3 ± 8.02^{a}
GCD[D]	1.33 ± 0.10^{a}	1.64 ± 0.37^{a}	2.46 ± 0.19^{b}	0.060 ± 0.007^{a}	33.3 ± 6.66^{a}
EXCD[E]	1.33 ± 0.08^{a}	1.70 ± 0.40^{a}	2.42 ± 0.13^{b}	0.054 ± 0.0011^{a}	25.9 ± 5.66^{a}

Values within the same column with different superscripts are significantly different ($p < 0.05$).
[A] Whole raw cowpea diet.
[B] Dehulled cowpea diet.
[C] Cooked cowpea diet.
[D] Germinated cowpea diet.
[E] Extruded cowpea diet.

Arteaga G, & Haard, 1999; Le Moullac, Klein, Sellos, & Van Wormhoudt, 1996; Rodríguez, Le Vay, Mourente, & Jones, 1994). The increase in trypsin activity, when vegetable ingredients are used in the diet, has been attributed to their trypsin inhibitor content, since it causes overproduction of this enzyme (Fennema, 1996). However, under the experimental conditions of our study, no significant correlation between the trypsin inhibitor activity in the hepatopancreas and the diets was found.

Chymotrypsin activity diminished as result of the inclusion of cowpea meal in the diets. Some studies have reported that the chymotrypsin activity in *L. vannamei* is not affected by the protein level in the feed, but that it can be influenced by the protein source (Le Moullac et al., 1994; Le Moullac et al., 1996). In mammals, there is a positive correlation between trypsin and chymotrypsin activities, due to the activation of chymortrypsinogens for the trypsin. Apparently, in crustaceans, this pattern is different, since no correlation between the activities of these enzymes was found.

4. Conclusions

The chemical composition of cowpea meals varies, depending on the process used for their elaboration. In general, cowpea meals can be considered as good sources of available protein and carbohydrates for *L. vannamei,* since their trypsin inhibitor activity is low and their digestibility is relatively high, especially in those meals treated thermally, such as cooked and extruded, which significantly improves the nutritional quality of cowpea for *L. vannamei*. Nevertheless, cowpea is deficient in some essential amino acids, such as methionine; hence, its use as a protein source in shrimp feeds is compromised, unless the feed is enriched with pure amino acids or combined with other protein sources. More research is needed to determine the optimal inclusion level of cowpea meals in the diet, and to assess their effect on shrimp survival and growth.

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