ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 7 (2): 36-46, 2013
ISSN 1819-1878 / DOI: 10.3923/ajas.2013.36.46

Digestibility of Different Thermal Processed Grain of Legumes, *Rynchosia minima* and *Cajanus cajan*, in white Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Since nutrition demands a high percentage of production costs in shrimp culture, the development of dietary strategies such as the inclusion of vegetable protein sources, is a very important tool. Thus, digestibility of alternative feedstuffs for evaluating its dietary potential as shrimp feed represents the first action. The objective of study was to determine digestibility coefficients for protein, energy, dry matter and amino acids of least snout bean and pigeon pea meals in Pacific white shrimp juveniles. Thirteen isonitrogenous diets (34.8 to 35% protein) were formulated and elaborated. One containing only fish meal as a source of protein and the other replacing 10 and 18% of fish meal with the leguminous seed meals *Rynchosia minima* and *Cajanus cajan*, previously subjected to a thermal process for 0, 45 and 90 min. The shrimp were fed to satiation four times daily for 25 day. The apparent digestibility coefficients were determined using 0.5% Cr_2O_3. Feces were collected from three replicate groups of shrimp. The results showed that shrimp fed with 10 and 18% *Cajanus cajan* and *Rynchosia minima* replaced and subjected to a thermal process of 45 min presented higher weight increase ($p<0.05$) and specific growth rate compared with control diet group. *L. vannamei* had relatively high apparent digestibility coefficients of crude protein (93.68 and 92.29%) and crude lipid (96.87 and 97.55%) for CC10-45 and RM18-45 diets, respectively. Results suggest that diets with both *R. minima* and *C. cajan* seed meals thermally processed, can partially replace animal protein in shrimp diets at inclusion levels of 10 and 18%, respectively.

Key words: Digestibility, shrimp, legumes, *L. vannamei*

INTRODUCTION

Various plant protein sources have been tested as source of pigments from plant extracts and their potential to partially replacement of fish meal in practical shrimp diets (Ponce-Palafox *et al.*, 2006). The legume seeds are characterized for contain high levels of energy and quality protein that could be useful as a component for practical diets; between they are include peas, grains and beans and other closely related species within the Fabaceae family (Vasagam *et al.*, 2007). According to Lim and Dominy (1990) and Samocha *et al.* (2004), soybean meal is the most widely vegetable protein source since its available worldwide, low price and nutritional content. Recently, the nutritionists had begging evaluated the suitability of grain legumes such as soybean products (Cabanillas-Beltran *et al.*, 2001; Cruz-Suarez *et al.*, 2009) feed pea (Cruz-Suarez *et al.*, 2001; Davis *et al.*, 2002; Bautista-Teruel *et al.*, 2003), lupin (Sudaryono *et al.*, 1999), cowpea and mung bean (Eusebio, 1991; Eusebio and Coloso, 1998; Vasagam *et al.*, 2007) in shrimp feeds. Grain legumes offer some advantages such as the flexibility in feedstuff selection to the feed manufacturer because they have the potential to provide both good energy and moderate protein to the diet.

The accurate measurement of nutrient digestion and assimilation efficiency of the dietary components, loosely termed digestibility, is essential to achieve an optimal diet formulation from both biologically and economically points of view. Akiyama *et al.* (1989) stated that feedstuff digestibility depends on its physical and nutritional characteristics, as well as other animal features and external factors such as digestive tract architecture and physiology and the environmental conditions, respectively. The potential of locally available legume seed meals obtained from Least snout bean (*Rynchosia minima*) and Pigeon pea (*Cajanus cajan*), which grow in north-western Mexico, as protein sources has been evaluated in *Litopenaeus vannamei* juveniles diets. Nevertheless, De Silva (1989) concluded that the nutritional bioavailabilities of such feed ingredients are often limited due to the presence of anti-nutritional factors and other toxic substances in legumes (Elias *et al.*, 1979; Siddhuraju *et al.*, 2002; Siddhuraju and Becker, 2005). There are some processing methods to inactivate or reduce the presence of antinutrients in plant feeds (Penaflorida, 1995; Siddhuraju and Becker, 2005), one of the most used is the application of a thermal process. The objective of the present study was to determine digestibility coefficients for protein, energy, dry matter and amino acids of least snout bean and pigeon pea meals in Pacific white shrimp juveniles *L. vannamei*.

MATERIALS AND METHODS

Diet preparation: Thirteen isonitrogenous diets (34.8 to 35% protein) were formulated and elaborated. Control Diet (CD) consisted in only Fish Meal (FM) as a source of protein and the other twelve replacing 10 and 18% of FM with the leguminous seed meals *Rynchosia Minima* (RM) and *Cajanus cajan* (CC). The proximate compositions of FM, RM and CC are presented in Table 1. Composition of the experimental diets and amino acid are shown in Table 2 and 3, respectively. The leguminous seed meals were subjected to thermal process (wet heat, 121°C) for 0, 45 and 90 min (RM10-0, RM18-0, CC10-0, CC18-0, RM10-45, RM18-45, CC10-45, CC18-45, RM10-90, RM18-90, CC10-90, CC18-90). The feeds were prepared in the laboratory by drying, grinding and passing them through a 0.5 mm screen mesh. A meat mincer with a 2.0 mm diameter die was used to produce the spaghetti-shaped diet that was cut in pieces and dried overnight at 40°C. After that, it was packed in black plastic bags and stored in a freezer at -20°C, until required.

Shrimp and experimental conditions: The Pacific white shrimp were selected for uniformity of size (10.0±0.47 g) from grow-out ponds at Thaimex farm, Nayarit, Mexico. The shrimps were randomly distributed into 15 chambers of 50 L with six shrimp in each one, with three repetitions by treatment. Each chamber was an adaptation of the Guelph method design

Table 1: Proximate composition of feedstuffs (percentage of total dry weight) used for formulating experimental diets

Parameters	Fish meal	*R. minima*	*C. cajan*
Moisture	7.05±0.05	5.92±0.9	5.87±0.8
Crude protein	57.72±0.07	22.59±0.1	18.80±0.6
Crude lipid	9.79±0.31	6.2±0.3	1.63±0.7
Crude fiber	1.50±0.05	11.12±0.1	7.09±0.4
Ash	14.23±4.14	4.15±1.2	3.44±3.7

Data represent mean±standard deviations of three replicates

Table 2: Ingredient composition of the reference diet used to measure digestibility (% as fed)

Ingredient	CD	RM10	RM18	CC10	CC18
Fish meal[a]	49.55	44.59	40.63	44.59	40.63
R. minima meal	0	18.9	34.02	0	0
C. cajan meal	0	0	0	20.49	34.72
Fish oil	5.8	5.72	4.61	4.92	4.8
Soybean oil	2.42	2.09	2.56	1.96	2.05
Starch	31.38	17.85	5.33	17.19	7.46
Mineral mix[b]	2	2	2	2	2
Vitamin mix[c]	3	3	3	3	3
Vitamin "C"[d]	0.6	0.6	0.6	0.6	0.6
Lecithin	0.25	0.25	0.25	0.25	0.25
Binder	4	4	4	4	3.99
Gelatin	0	0	2	0	0
Cholesterol	0.5	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Chemical composition (g/100 dry matter) of the diets used to determine *in vivo* digestibility					
Crude protein	36.0±5.2	35.9±6.3	35.8±4.8	35.9±2.0	35.8±1.4
Crude lipid	8.7±2.9	9.1±3.3	9.2±2.7	9.3±3.9	9.4±2.2
Gross energy (Kj g^{-1})	18.5±1.1	18.4±0.9	18.5±1.3	18.7±0.7	18.1±0.8

[a]Sardine meal 57% CP. Alimentos marinos, Ciudad Obregón, Sonora, México. [b]Mineral mixture composition: Co, 2 g kg^{-1}; Mn, 16 g kg^{-1}; Zn, 40 g kg^{-1}; Cu, 20 g kg^{-1}; Fe, 1 mg kg^{-1}; Se, 100 mg kg^{-1}; I, 2 g kg^{-1}. [c]Vitamin mixture composition: retinol, 4000 IU g^{-1}; thiamin, 24 g kg^{-1}; riboflavin,16 g kg^{-1}; DL Ca pantotenate, 30 g kg^{-1}; pyridoxine, 30 g kg^{-1}; cyanocobalamin, 80 mg kg^{-1}; ascorbic acid, 60 mg kg^{-1}; menadione, 16 mg kg^{-1}; cholecalciferol, 3200 IU g^{-1}; tocopherol, 60 g kg^{-1}; biotin, 400 mg kg^{-1}; niacin, 20 mg kg^{-1}; folic acid, 4 g kg^{-1}. [d]Vitamin C (L-ascorbyl-2-polyphospate, 35%)

(Martinez *et al.*, 2001). Water was supplied to each experimental system after biological and mechanical filtration, temperature control (±1°C) and aeration. Daily measurements of temperature, salinity (Atago refractometer), pH (Corning 56 pH-meter) and dissolved oxygen (YSI 59 D.O. meter) were taken in tanks selected at random. Ammonia, nitrite and nitrate (Strickland and Parsons, 1977) were measured in chambers, three times a week. Dechlorinated tap water was added to the biofilters to replace evaporation losses, as required. Temperature, salinity, pH, nitrite, nitrate and total ammonia nitrogen were maintained at 27.0°C, 35‰, 8.0±0.3, 0.49±0.14, 16.1±4.8 mg L^{-1} and 0.03 mg L^{-1}, respectively, while dissolved oxygen did not fall below 6.0 mg L^{-1}.

Evaluation of growth parameters: Shrimp production was evaluated at the end of the growth trial, considering the following parameters: weight gain, feed conversion ratio, survival, protein efficiency ratio, specific growth rate (Goytortua-Bores *et al.*, 2006). The weights of the shrimp were recorded at 0 (IBW) and 25 (FBW) day.

Table 3: Amino acid composition (g/100 g crude protein) of experimental diets

Diet	Histidine	Arginine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenylalanine	Lysine
Essential amino acids									
CD	1.02±0.14	2.05±0.31	1.30±0.18	1.29±0.19	0.96±0.13	1.36±0.19	2.53±0.37	1.43±0.19	3.67±0.46
RM10-0	0.90±0.09	1.76±0.14	1.47±0.13	1.41±0.11	0.73±0.05	1.23±0.06	2.30±0.03	1.29±0.12	2.53±0.08
RM18-0	0.69±0.16	1.70±0.03	1.51±0.08	1.69±0.01	0.77±0.06	1.42±0.002	2.52±0.07	1.37±0.06	2.31±0.05
RM10-45	0.78±0.04	1.48±0.03	1.24±0.02	1.33±0.04	0.69±0.004	1.15±0.02	2.32±0.07	1.12±0.05	2.65±0.06
RM18-45	0.73±0.03	1.45±0.05	1.20±0.03	1.19±0.04	0.60±0.01	1.00±0.02	2.03±0.04	1.02±0.03	2.27±0.12
RM10-90	0.87±0.01	1.74±0.01	1.46±0.03	1.47±0.04	0.71±0.01	1.33±0.005	2.63±0.05	1.31±0.03	2.37±0.22
RM18-90	0.94±0.15	1.43±0.09	1.41±0.16	1.38±0.02	0.57±0.14	1.17±0.06	2.22±0.10	1.22±0.06	2.57±0.13
CC10-0	0.78±0.03	1.51±0.09	1.31±0.08	1.15±0.07	0.64±0.03	1.04±0.06	1.88±0.15	1.19±0.01	2.22±0.24
CC18-0	0.88±0.09	1.47±0.10	1.31±0.13	1.33±0.13	0.66±0.10	1.21±0.12	2.41±0.30	1.43±0.19	2.53±0.28
CC10-45	0.75±0.03	1.46±0.06	1.20±0.10	1.30±0.07	0.70±0.03	1.08±0.07	1.95±0.13	1.19±0.07	1.95±0.17
CC18-45	0.83±0.04	1.39±0.07	0.97±0.33	1.20±0.07	0.61±0.04	1.05±0.05	2.20±0.11	1.15±0.12	2.41±0.17
CC10-90	0.87±0.11	1.46±0.17	1.33±0.17	1.29±0.16	0.61±0.07	1.10±0.16	2.30±0.34	1.25±0.12	2.53±0.37
CC18-90	0.76±0.09	1.53±0.17	1.35±0.19	1.43±0.19	0.79±0.11	1.20±0.19	2.15±0.31	1.10±0.15	1.92±0.30

Diet	Aspartic acid	Glutamic acid	Serine	Glycine	Alanine	Proline	Tyrosine
Non-essential amino acids							
CD	3.13±0.53	5.14±0.84	1.08±0.16	1.02±0.10	1.35±0.18	1.18±0.13	1.09±0.18
RM10-0	2.80±0.45	4.47±0.54	1.47±0.21	1.95±0.33	1.98±0.20	1.65±0.18	0.95±0.07
RM18-0	3.13±0.002	4.78±0.02	1.59±0.04	1.72±0.07	2.00±0.04	1.51±0.02	1.06±0.05
RM10-45	2.79±0.21	4.23±0.29	1.28±0.07	1.53±0.01	1.71±0.03	1.31±0.04	0.82±0.01
RM18-45	2.29±0.19	3.75±0.09	1.23±0.02	1.72±0.24	1.61±0.10	1.53±0.09	0.78±0.03
RM10-90	3.16±0.05	4.73±0.06	1.49±0.03	1.92±0.09	1.97±0.03	1.78±0.14	0.93±0.03
RM18-90	2.81±0.12	4.36±0.17	1.47±0.12	1.70±0.01	1.57±0.11	1.88±0.42	0.86±0.12
CC10-0	2.33±0.35	4.15±0.41	1.33±0.02	2.04±0.28	1.72±0.09	1.47±0.05	0.81±0.07
CC18-0	2.69±0.24	4.66±0.56	1.35±0.09	1.68±0.13	1.83±0.09	1.33±0.03	0.77±0.11
CC10-45	2.51±0.21	4.13±0.21	1.25±0.07	1.57±0.05	1.66±0.06	1.23±0.05	0.80±0.06
CC18-45	2.76±0.20	4.48±0.28	1.26±0.08	1.35±0.09	1.58±0.10	1.18±0.06	0.74±0.03
CC10-90	2.82±0.34	4.51±0.56	1.31±0.17	1.54±0.18	1.68±0.19	1.36±0.18	0.71±0.08
CC18-90	2.86±0.34	4.47±0.51	1.35±0.17	1.64±0.22	1.84±0.24	1.25±0.18	0.84±0.10

Values are means of three samples±SD

Fecal collection techniques: During the experiment, the chambers were covered with black net mesh to reduce the effects of the stress caused by the disturbance of the laboratory staff passing-by. Groups of five animals (10.0±0.46 g) were randomly selected for each diet and transferred into the experimental arrays where they were acclimated during eight days. Before each feeding procedure, all the faecal material was removed. The shrimp were fed with the experimental diets to visual satiety four times a day at 08:00, 12:00, 16:00 and 20:00 h. Feces were removed 1 to 4 h after each feeding. At each feeding, shrimp were given 1 to 4 h to consume a measured quantity of diet after which feces and uneaten diet in each chamber were collected from the settling trap into a sample bottle. Then, faecal samples were then gently rinsed with distilled water and dried overnight on Petri dishes at 105°C. Feces collections were terminated after 25 d, when approximately 10 g of dry feces had been collected from shrimp fed in each of the treatments.

Chemical analysis: Diets and fecal samples were freeze-dried and stored at -20°C until used for chemical analysis. Crude protein was determined by Kjeldahl method using an Auto Kjeldahl System (Tecator, 1030). Crude lipid was determined by the Soxhlet extraction method (Soxtec 2050 FOSS Model, Switzerland) and ash content by a furnace muffler (Naberthern, model K, Germany) at 550°C for 4 h. Fiber crude was analyzed according to AOAC (1995). Moisture was determined by oven drying at 105°C for 24 h (AOAC, 1995). The gross energy of the diets and feces was calculated according to the NRC (1993) procedure.

Amino acid composition: Amino acids were determined after hydrolysis of samples (fecal and diets) in 6 N HCl for 24 h at 110°C. Then, the samples were evaporated to dryness, mixed with Na-EFDR buffer and then analysed using a High Performance Amino Acid (Beckman System 6300). Tryptophan concentration was not determined.

Apparent digestibility: Nutrient apparent digestibility was determined collecting the feces during 25 d by settling trap into a sample bottle. Fecal samples were rinsed with distilled water to remove the excess of salts and frozen (-20°C). Chromic oxide was added to the samples of diets as an index of the undigested compound. The percentage of utilization was calculated from the concentrations of this index in the feed and the excreta (McGinnis and Kasting, 1964). Chromic oxide levels, both in diets and faeces, were determined by first digesting the organic matter with nitric acid and then oxidizing Cr_2O_3 to Cr_2O_7 with perchloric acid followed by the colorimetric analysis of the dichromate ion with diphenylcarbazide (Furukawa and Tsukahara, 1966). All chemical determinations were made on a dry-weight basis and in triplicate.

Statistical analysis: Percent data were transformed to the arcsine of their square root prior to analysis. All data were subjected to one-way analysis of variance to test the effects of experimental diets using the STATISTICA 6.0 software package (Statsoft, Tulsa, OK, USA). Bartlett's chi-squared test was used for homogeneity test and there were no differences among variances. Multiple comparisons among means were made by ANOVA and using Tukey's test was used to test differences among individual means. Difference was regarded as significant when p was <0.05 (Montgomery, 2005).

RESULTS

Growth indices: At the end of the trial, the experimental diet CC10-45 exhibited higher values of FBW, WG, SGR and PER, in contrast with diet CC10-90 which presented lower values in all five-growth performance indicators evaluated in shrimp juvenile. The diets RM10-45, RM18-45 and CC18-45 also showed at least three higher values (Table 4). The shrimp fed with the RM10-90, CC10-45 and CC18-90 showed significantly higher Protein Efficiency Ratio (PER). Finally the shrimp survival rate was 100% in all treatments.

Nutrient digestibility: Results of the digestibility study showed that the Apparent Digestibility Coefficients (ADC) of the dry matter (ADDM) in shrimp fed with RM18-45, CC10-45 and CC18-45 were significantly higher than that obtained for the CD diet (Table 5). ADCs of ADDM, ADCP and ADCL decreased with thermal process of 90 min. Significant decrease in apparent digestibility for crude protein (ADDM, from 90.51 to 71.54%), crude protein (92.22 to 83.02%) and crude lipid (ADCL, from 97.55 to 92.45%) was observed. Shrimp fed CC10-45 diet showed significantly ($p<0.05$) higher ADDM (90.51%) and ADCP (93.68%).

Table 4: Growth performance of juvenile *L. vannamei* fed with different levels of CC and RM

Diet	IBW (g)	FBW (g)	FC	WG (g/week)	SGR (%/d)	PER
CD	9.87^{a}	14.71^{b}	1.48^{b}	1.04^{b}	1.60^{b}	1.93^{a}
RM10-0	9.91^{a}	13.62^{b}	1.63^{b}	1.04^{b}	1.27^{c}	1.75^{b}
RM18-0	10.02^{a}	13.88^{b}	1.72^{b}	1.08^{b}	1.30^{c}	1.66^{b}
RM10-45	10.13^{a}	15.32^{a}	1.81^{a}	1.45^{a}	1.65^{b}	1.58^{c}
RM18-45	9.89^{a}	15.51^{a}	1.56^{b}	1.57^{a}	1.80^{a}	1.83^{b}
RM10-90	9.88^{a}	13.61^{b}	1.29^{c}	1.04^{e}	1.28^{c}	2.21^{a}
RM18-90	9.80^{a}	13.74^{b}	1.88^{a}	1.10^{b}	1.35^{c}	1.52^{c}
CC10-0	10.12^{a}	14.35^{b}	1.92^{a}	1.18^{b}	1.40^{d}	1.49^{c}
CC18-0	10.15^{a}	14.8^{b}	1.90^{a}	1.30^{b}	1.51^{b}	1.50^{c}
CC10-45	10.19^{a}	16.44^{a}	1.32^{c}	1.75^{a}	1.91^{a}	2.16^{a}
CC18-45	9.87^{a}	15.27^{a}	1.59^{b}	1.51^{a}	1.75^{a}	1.80^{b}
CC10-90	10.12^{a}	14.01^{b}	1.67^{b}	1.09^{b}	1.30^{c}	1.71^{b}
CC18-90	10.09^{a}	14.22^{b}	1.39^{c}	1.16^{b}	1.37^{c}	2.06^{a}

Values in the same column with different superscript letters are significantly different ($p< 0.05$). (Tukey's test; $p< 0.05$); IBW: Initial body weight, FBW: Final body weight, FCR (feed conversion ratio): Total dry feed offered (g)/total wet weight gain (g), WG: Weight gain, SGR: Specific Growth Rate, PER (Protein efficiency ratio): (shrimp weight gain, g)/(protein intake, g)

Table 5: Apparent digestibility coefficients (ADC,%±SD) for dry matter (ADDM), crude protein (ADCP) and crude lipid (ADCL) in experimental diets consumed by *L. vannamei* (means; n = 3)

Treatment	ADDM	ADCP	ADCL
CD	86.17±0.14^{a}	85.05±0.09^{b}	95.46±0.15^{a}
RM10-0	75.65±0.52^{c}	84.74±0.42^{b}	92.45±0.12^{b}
RM18-0	68.61±0.46^{d}	84.72±0.08^{b}	92.83±0.44^{b}
RM10-45	85.09±0.15^{a}	86.07±0.29^{b}	93.53±0.50^{b}
RM18-45	80.82±0.19^{b}	91.29±0.32^{a}	97.27±0.15^{a}
RM10-90	74.74±0.33^{c}	85.61±0.46^{b}	96.43±0.30^{a}
RM18-90	73.11±0.27^{c}	85.80±0.17^{b}	95.05±0.67^{a}
CC10-0	84.98±0.12^{a}	84.79±0.13^{b}	95.69±0.20^{a}
CC18-0	85.89±0.38^{a}	84.78±0.28^{b}	94.88±0.08^{a}
CC10-45	90.51±0.17^{a}	93.68±0.16^{a}	96.87±0.16^{a}
CC18-45	88.71±0.09^{a}	92.22±0.22^{a}	97.55±0.53^{a}
CC10-90	75.83±0.45^{c}	83.34±0.50^{c}	93.96±0.29^{b}
CC18-90	71.54±0.21^{c}	83.02±0.35^{c}	93.98±0.47^{b}

R: *Rynchosia minima,* C: *Cajanus cajan,* Thermal process = 0, 45 and 90°C; Inclusion in the diet = 10 and 18%. Values in the same column with different superscript letters are significantly different. (Tukey's test; $p< 0.05$)

Table 6: Apparent digestibility coefficients (ADC,%) of amino acids in various CC and RM treated diets and difference in digestibility of amino acids and crude protein consumed by *L. vannamei* shrimp

Diet	Histidine	Arginine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenylalanine	Lysine
Essential amino acids									
CD	93.91[a]	90.92[a]	91.40[a]	91.66[a]	92.85[a]	92.21[a]	94.22[a]	90.73[a]	95.91[a]
RM-0-10	82.61[c]	89.49[b]	81.80[b]	81.64[b]	81.25[b]	81.59[c]	82.70[b]	88.72[b]	86.58[b]
RM-0-18	81.07[c]	76.56[c]	83.73[b]	85.16[b]	84.51[b]	84.11[b]	86.28[b]	75.20[d]	84.93[b]
RM-45-10	87.50[b]	81.39[c]	86.51[b]	87.61[b]	84.46[b]	86.28[b]	89.93[a]	86.66[b]	90.75[a]
RM-45-18	93.60[a]	92.51[a]	92.65[a]	93.61[a]	92.01[a]	93.71[a]	94.69[a]	89.55[b]	95.44[a]
RM-90-10	90.78[a]	87.15[b]	90.88[a]	91.64[a]	91.63[a]	91.82[a]	93.25[a]	87.88[b]	95.17[a]
RM-90-18	88.00[b]	80.71[c]	85.61[b]	86.14[b]	80.49[b]	85.57[b]	87.06[c]	84.90[b]	91.07[a]
CC-0-10	86.22[b]	77.84[c]	84.59[b]	83.42[c]	82.16[b]	84.34[b]	86.28[b]	86.84[b]	88.90[b]
CC-0-18	85.23[b]	82.57[c]	84.83[b]	84.66[a]	82.77[b]	84.14[b]	85.93[b]	83.09[b]	86.51[b]
CC-45-10	94.61[a]	93.27[a]	94.45[a]	94.68[a]	93.16[a]	94.42[a]	94.76[a]	91.23[a]	94.72[a]
CC-45-18	93.82[a]	91.84[a]	90.68[a]	92.01[a]	92.25[a]	93.03[a]	94.33[a]	90.44[a]	95.93[a]
CC-90-10	82.85[c]	72.58[d]	83.25[b]	83.85[c]	71.35[c]	83.14[b]	86.01[b]	79.66[d]	86.08[b]
CC-90-18	84.29[c]	83.43[c]	87.41[b]	88.31[b]	84.48[b]	87.91[b]	88.66[b]	76.44[d]	87.76[b]

Diet	Aspartic acid	Glutamic acid	Serine	Glycine	Alanine	Proline	Tyrosine
Non-Essential amino acids							
CD	96.75[a]	95.55[a]	88.22[b]	88.60[b]	91.57[a]	89.18[a]	90.10[a]
RM-0-10	82.14[b]	82.18[b]	81.74[c]	84.52[b]	84.53[b]	82.50[b]	89.26[a]
RM-0-18	80.11[b]	88.18[b]	84.69[c]	80.49[c]	87.64[b]	83.31[b]	77.06[c]
RM-45-10	93.94[a]	91.25[a]	87.03[b]	88.39[b]	90.84[a]	84.65[b]	74.16[c]
RM-45-18	94.15[a]	94.03[a]	92.82[a]	94.86[a]	95.11[a]	93.73[a]	90.39[a]
RM-90-10	93.86[a]	91.96[a]	90.90[a]	92.67[a]	93.54[a]	91.46[a]	89.15[a]
RM-90-18	92.77[a]	89.94[a]	86.91[b]	89.86[b]	89.03[a]	89.14[a]	80.11[b]
CC-0-10	81.24[b]	89.42[a]	83.39[c]	89.77[b]	88.95[b]	84.34[b]	77.03[c]
CC-0-18	86.67[b]	86.95[b]	84.60[c]	86.25[b]	86.33[b]	85.02[b]	80.01[b]
CC-45-10	97.88[a]	96.48[a]	94.49[a]	96.09[a]	95.85[a]	94.18[a]	93.54[a]
CC-45-18	95.91[a]	95.30[a]	92.88[a]	93.70[a]	94.61[a]	92.51[a]	88.54[a]
CC-90-10	92.37[a]	87.24[b]	82.84[c]	85.73[b]	87.66[b]	81.98[b]	65.56[d]
CC-90-18	95.57[a]	92.14[a]	86.34[b]	90.60[a]	90.73[a]	85.28[b]	84.82[b]

In the column, different letters mean statistical difference at $p<0.05$

Digestibility of amino acids: Experimental diets CD, CC45-10, and CC45-18 presented higher values of essential amino acids followed by CC45-10 and CC45-18 and presented significantly differences ($p<0.5$) with respect to the other diets. Diets RM45-18, RM90-10, CC45-10 and CC45-18 showed higher values of non-essential amino acids (Table 6).

All data found in this research indicated that diet containing 10% of CC and treated with thermal process by 45 min, resulted be the best experimental diet to fed shrimp juvenile, such as was show by the growth performance indicators. Moreover, this diet presented higher Apparent Digestibility Coefficients (ADC) of the dry matter (ADDM) and higher values of essential amino acids.

DISCUSSION

The determination of nutrient digestibility is the first step for evaluating the potential of an ingredient to be utilized in animal feed (Allan *et al*., 2000; Luo *et al*., 2012). Bioavailability of proteins and amino acids in feedstuffs is an important factor to contemplate, because they are related with the quantity of nitrogen absorbed by shrimp (Terrazas *et al*., 2010). During high density cultivation, almost 78% of nitrogen derived from dietary protein is released to the environment (Jackson *et al.*, 2003). The chemical composition of the experimental diets tested in this research was equalled in protein and energy and at levels supposed to be optimal for pacific white shrimp (Amaya *et al.*, 2007; Oujifard *et al.*, 2012). All diets fed to shrimp in the present study had similar amino acid values similarly to those reported by Oujifard *et al.* (2012) for *L. vannamei*. Final body weight showed no differences by increasing the dietary levels of RM and CC up to 18% and subjected to a thermal process of 45 min, 0 and 90 min thermal process induced a growth reduction about 13% (Table 4). Survival, however, was not affected indicating that the juvenile shrimps are less sensitive to dietary EM and CC level. The results are considered good compared with those reported for *Penaeus monodon* fed the lupin meal, cow pea and mung bean (Sudaryono *et al.*, 1999; Vasagam *et al.*, 2007) and *L. vannamei* fed the cow pea, full fat soybean meal, solvent extracted soybean meal, soybean protein concentrate, soybean protein isolate and rice protein concentrate, (Cruz-Suarez *et al.*, 2009; Oujifard *et al.*, 2012). The digestibility of a feed ingredient depends primarily on its chemical composition and the digestive capabilities of the shrimp to which it is fed. However, factors unrelated to diet formulation such as environmental conditions in the production system, feeding practices and diet manufacturing techniques affect the digestibility. However, digestibility coefficients are not often constant (McGoogan and Reigh, 1996).

Antinutritional factors ANFs were significantly reduced by thermal process, since digestibility with tested diets was inferior when compared with diets without thermal process. The improvement in digestibility due to the thermal process treatments might be attributed to disruption of protein structures and cell wall-encapsulated starch, starch gelatinisation and physical disintegration of the legume seeds. Also, thermal process is found to be most effective among the different processing methods for the reduction of various antinutrients in legume seeds (Rehman and Shah, 2005; Siddhuraju and Becker, 2005).

The improved growth performance in the shrimp fed with CC10-45 might be due to thermal process time of the diet. In general, there are several reasons for the reduced growth rates among treatments, from which the decreased ADC can be the most important. When alternate plant protein sources are used in diets containing the same concentration of digestible energy and protein and are able to meet the nutritional requirements of the animal being fed, similar growth may be expected (Cruz-Suarez *et al.*, 2001).

In the present study, ADCs of ADCP and ADCL decreased progressively from CC10-45 to CC18-0 (Table 5). The ADCP (93.7%) and ADDM (90.5%) from up to 10% CC10-45 inclusion of the present study (Table 5), are comparable with the mean values of ADCP (87.9%) and ADDM (69.9%) reported for *P. monodon* fed lupin protein concentrate, cow pea and mung bean (Sudaryono *et al.*, 1999; Vasagam *et al.*, 2007) and with the mean values of ADCP (96.9%) and ADDM (91.7%) reported for *L. vannamei* fed four soybean ingredients (Cruz-Suarez *et al.*, 2009). Digestibility of protein was significantly affected by the thermal process, followed by reduced digestibility of most amino acids in the diets.

Generally, the decreasing trend in ADC for AA was observed when the thermal process was not applied and level increase of 45 to 90. The ADCAA of the up to 18% CC and RM included diets ranged from 81.25% (excluding serine) to 94.68% (Table 6) which are similar to those reported by Cruz-Suarez *et al.* (2001) (86-95%) and Oujifard *et al.* (2012). Arginine and serine showed the lower apparent digestibility whereas lysine and aspartic acid obtained the higher digestibility in all diets.

In general, it was found that the digestibility of *C. cajan* was consistently higher than that of *R. minima*, in terms of ADCDM and ADCCP, suggesting that *C. cajan* meal is more easily digested. Compared to the values reported by Akiyama *et al.* (1989) and Cabanillas-Beltran *et al.* (2001) for

L. vannamei, the ADCDM, ADCCP and ADCCL values obtained in this study for *C. cajan* and *R. minima* were consistently higher. The high protein digestibility of plant origin feedstuffs may be related to the omnivorous/herbivorous feeding habit of *L. vannamei*. The AAAD values obtained for *C. cajan* and *R. minima* were lower than those of Akiyama *et al.* (1989) and Cabanillas-Beltran *et al.* (2001). The results suggest that *R. minima* seed meal (RM45-18) or *C. cajan* seed meal (CC45-10) diets can partially replace animal protein in shrimp diets at inclusion levels of 10 and 18%, respectively.

CONCLUSION

This study showed that whole *C. cajan* and *R. minima* meal is a very acceptable ingredient for white shrimp diets; thermal process improved digestibility. Results suggest that diets with both *R. minima* and *C. Cajan* seed meals thermally processed, can partially replace animal protein in shrimp diets at inclusion levels of 10 and 18%, respectively.

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