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Evaluation of amaranth (*Amaranthus caudatus* L.) and quinoa (*Chenopodium quinoa*) protein sources as partial substitutes for fish meal in *Litopenaeus vannamei* grow-out diets

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Abstract

Two groups of isonitrogenous diets formulated by replacing 15%, 25%, 35% and 45% of fish meal protein by amaranth meal and quinoa meal were used to evaluate the performance of *Litopenaeus vannamei*. Growth showed significant reduction ($P < 0.05$) in the group of shrimp fed with amaranth diets, with diet A15 showing the best specific growth rate (SGR = 2.81% day^{-1}), but after the control diet AQ0 (3.07% day^{-1}). Diet A15 had significantly ($P < 0.05$) the best digestibility of dry matter (79.7%) and protein (88.4%) without differences compared to control diet AQ0 (75.1% and 85.2%). Replacement with quinoa meal at any level tested did not significantly affect ($P > 0.05$) the shrimp growth performance. Shrimp fed with quinoa diets showed better SGR (3.05% day^{-1}) than those shrimp fed with amaranth (2.56% day^{-1}). No differences in feed conversion ratio appeared in either of the protein sources, but quinoa diets presented a better average (3.13) than amaranth diets (4.01). The apparent digestibility of dry matter and protein for quinoa diets was similar for all diets, but they were statistically different ($P < 0.05$) from the control diet. We conclude that quinoa meal can replace fishmeal up to 45%, whereas it can be replaced with amaranth meal up to 15%, without adverse effects on growth and survival.

Keywords: plant protein, shrimp growth, digestibility, fishmeal replacement

Introduction

Efficient production of aquatic species in intensive and semi-intensive production systems requires the use of commercial feed as a primary or supplementary source of nutrients respectively. Fishmeal is an animal protein source commonly used in feeds for aquatic animal and it is the main ingredient in commercially manufactured feeds due to its high palatability and good nutritional balance (Sudaryono, Tsvetnenko & Evans 1999). Inclusion levels of fishmeal in commercial diets (CD) vary from 100 to 500 g kg^{-1} (Tacon & Metian 2008). This reliance on the use of fishmeal as the main protein component in formulated diets is a factor that significantly affects operating costs of shrimp farming. In contrast to the production of fishmeal, aquaculture is in constant growth; so other protein sources will have to be identified and evaluated for use in feeds for fish and shrimp.

Commercial shrimp feeds contain 300–500 g kg^{-1} crude protein, which comes mainly from animal products such as marine fish, shrimp and squid (Mente, Coutteau, Houlihan, Davidson & Sorgeloos 2002). The need to seek new protein sources, through evaluation of plant or animal ingredients in terms of growth response and nutrients digestibility in order to incorporate them into feeding of different shrimp species (Shiau 2008; Venero, Davis & Lim 2008), is a long-standing issue. It is clear that most attention has been focused on studies of soybean meal as a substitute for animal protein (Dersjant-Li 2002) due to its

nutritional quality, low cost and constant availability (Mbahinzireki, Dabrowski, Lee, El-Saidy & Wisner 2001). An earlier work showed that soybean meal could effectively replace up to 42% of fish meal protein in feeds for *L. vannamei* (Lim & Dominy 1990, 1992), but when soybean was co-extruded with poultry by-products it can replace up to 80% of fish meal without having adverse effect on survival and feed conversion (Davis & Arnold 2000). Many researchers have tried to replace the expensive fish meal as ingredient in feeds for shrimp *L. vannamei* feed with other available protein sources such as cottonseed (Lim 1996), canola meal (Lim, Beames, Eales, Prendergast, Mclesse, Shearer & Higgs 1997), pea meal (Davis, Arnold & McCallum 2002; Martínez-Rocha, Gamboa-Delgado, Nieto-López, Ricque-Marie & Cruz-Suarez 2012), barley-based fermented grains (Molina-Poveda & Morales 2004), a mixture of plant and poultry by-products (Amaya, Davis & Rouse 2007a,b), algae (Hanel, Broekman, de Graaf & Schnack 2007; Ju, Forster & Dominy 2009), a mixture of plant protein soybean, canola and milo (sorghum) (Suárez, Gaxiola, Mendoza, Cadavid, Garcia, Alanis, Suárez, Faillace & Cuzon 2009; Sookying & Davis 2011), Jatropha kernel meal (Harter, Buhrke, Kumar, Focken, Makkar & Becker 2011), a mixture of meat, poultry, blood and corn gluten meal (Ye, Wang, Li, Sun & Liu 2011; Ye, Liu, Kong, Wang, Sun, Zhang, Zhai & Song 2012), soy protein concentrate (Bauer, Prentice-Hernandez, Borges, Wasielesky & Poerch 2012; Sá, Sabry-Nieto, Cordeiro-Junier & Nunes 2012), rice protein concentrate (Oujifard, Seyfabadi, Kenari & Rezaei 2012), lupin meal (Molina-Poveda, Lucas & Jover 2013) and corn gluten meal (Molina-Poveda, Lucas & Jover 2014).

Among other plant species with high nutritional value is *Amaranthus* spp., which is widely cultivated in Ecuador as a minor fodder crop in the highlands. The most common amaranth seed contains 145–160 g kg^{-1} crude protein (Table 1), about twice that of cereal grains, and also has a high lysine content which makes it particularly attractive to increase the biological value of processed feeds (Pedersen, Hallgren, Hansen & Eggum 1987). As for carbohydrates, the seed contains 580–680 g kg^{-1} starch, about 200 g kg^{-1} amylose and 50 g kg^{-1} sugar. Starch granules are extremely small (1–3 μm) and gelatinized at 55–65°C, giving unique gelatinization characteristics which may be of benefit for the food industry (Lehman 1988).

There are a few reports on the use of amaranthus in aquafeeds. Virk and Saxena (2003) studied amaranthus seeds as replacement for rice bran and groundnut oil cake at three different levels (20%, 35%, and 50%) in diets for common carp, *Cyprinus carpio*, and rohu, *Labeo rohita*, under a semi-intensive production system. Growth in terms of body weight gain was maximum in fish fed on diets containing 200 g kg^{-1} amaranthus seeds. Overall, the fish fed on diets containing amaranthus seeds at different levels showed better growth than the control. Adewolu and Adamson (2011) evaluated amaranthus leaf meal as dietary protein source in diets for *Clarias gariepinus*. *Amaranthus spinosus* leaf meal was included in the diets at 0, 50, 100, 150 and 200 g kg^{-1} and delivered to fingerlings of 5 g for 56 days. The results indicated that up to 50 g kg^{-1} *A. spinosus* leaf meal could be included in practical diets for *C. gariepinus* without affecting growth and feed utilization.

Quinoa (*Chenopodium quinoa*) is an ancient agricultural species originated from South America, which has been cultivated as a protein source in human nutrition for 7000 years (Jacobsen 2003). Quinoa production has increased in the last

Table 1 Proximate composition (g kg^{-1}) of ingredients and amino acid profile (g kg^{-1} protein) of amaranth, quinoa and fish meal used in feed formulation on dry matter basis

	Amaranth	Quinoa	Fishmeal
Moisture	38.3	95.9	64.6
Crude protein ($N \times 6.25$)	174.7	154.4	703.0
Crude lipid	64.9	83.4	120.5
Crude fibber	13.8	9.1	
Ash	28.2	25.3	117.5
Calcium	0.9	0.6	19.6
Phosphorous	7.4	7.3	19.4
Energy (MJ kg^{-1})	200.4*	201.2*	203.8
Amino acids			
Arginine	100.0	74.0	54.7
Histidine	25.0	46.0	24.6
Isoleucine	37.0	70.0	36.9
Leucine	57.0	73.0	73.8
Lysine	80.0	84.0	80.7
Methionine	42.0	55.0	30.1
Phenylalanine	77.0	53.0	39.6
Threonine	36.0	57.0	43.7
Valine	43.0	76.0	47.8

*Peralta, Mazón, Murillo, Villacrés, Rivera and Subia (2009).

40 years. The main producing countries are Bolivia, Peru and Ecuador, which in 2011 produced 80 255 tons, from 17 747 tons in 1971 (FAOSTAT 2011). During 2011, quinoa production was 41 182 tons in Peru; 38 257 tons in Bolivia; and 816 tons in Ecuador (FAOSTAT 2011).

Quinoa is an annual plant that grows in the Andean regions in a wide range of extraordinary *altiplano* conditions (FAO 2011). The grain is small, and typically has a protein content of 140–180 g kg^{-1} when compared to 100–120 g kg^{-1} for major cereals. Quinoa is particularly rich in essential amino acids (EAA), which are scarce in other cereals (FAO 2011), possessing higher proportions of lysine and the sulphur-containing amino acids, cysteine and methionine.

As in amaranth, the starch is mainly located in the perisperm and occurs at 60% in quinoa as compound granules of average particle size of 0.8–1.5 μm in different native varieties of Ecuador, having an amylose content of less than 110 g kg^{-1} (Koziol 1992). Triterpenoid saponins have been detected in quinoa and considered to be toxic to fish, limiting the inclusion levels of several alternate plant protein sources in diets for fish (Francis, Makkar & Becker 2001). Quinoa is also a source of a wide range of vitamins and minerals and is particularly high in iron (Repo-Carrasco, España & Jacobsen 2001). In a digestibility study on tilapia reported by Gutiérrez-Espinosa, Yossa-Perdomo and Vásquez-Torres (2011), these authors working with tilapia nilotica found that the dry matter, protein and energy apparent digestibility (AD) coefficients were, respectively, 58.8–64.4%, 67.7–77.5% and 29.0–66.1%, similar to other cereals.

The aim of this study was to determine if fishmeal can be replaced by other protein sources such as amaranth and quinoa in experimental diets for juveniles of the shrimp *Litopenaeus vannamei*, while maintaining the dietary protein and lipid content constant. The diets were evaluated in aquaria under controlled conditions in terms of digestibility, ingestion, growth and survival rates of shrimps.

Material and methods

Quinoa saponin removal

The seeds of quinoa *C. quinoa* were rinsed in clean water three times, thereby withdrawing the shell and therefore the saponins, which constitute an antinutritional factor. The seeds were then drained and left to dry in an oven for 24 h at 60°C. Like other ingredients, quinoa and amaranth were pulverized to 300 μm.

Experimental diets

Two sets of experimental diets were prepared with a level of 300 g kg^{-1} crude protein, including a control diet in which fish meal was replaced by vegetable protein 150, 250, 350 and 450 g kg^{-1}, the first group by amaranth meal and the second one by quinoa meal. In addition, a CD (326 g kg^{-1} protein and 72 g kg^{-1} lipid) was tested.

Experimental diets were prepared by weighing ingredients (0.01 g) and mixing them by hand from low to higher concentration. Vitamins and minerals were premixed before incorporation with the other ingredients. Once the dry ingredients were homogenized, lecithin was added followed by the fish oil. As a final step, hot water was added gradually until dough is obtained that could be processed in a meat grinder. For the digestibility trial, chromic oxide was added and blended previously with a portion of diet to ensure a homogeneous distribution of this marker before running the batch through a mincer with a 2 mm matrix. The noodles were dried in a vertical fan oven ISUZU (Type MNS 1155; Tokyo, Japan) at 60°C for 2 h and then strands were broken into pellets about 5–6 mm in length. All experimental feeds were stored and packaged in plastic bags at −10°C until use.

The lipid level of 65 g kg^{-1} diet was adjusted by varying the level of fish oil and the diet formulas were adjusted to 100% with corn starch. The composition of diets are presented in Table 2.

Palatability of diets

Acceptability was estimated based on the ingestion rate determined over 6 days; for which 2 h after each feeding, at 08:00 and 16:00 hours, the uneaten pellets (no faeces) were collected by siphoning in mesh of 300 μm previously weighed. Meshes containing the pellets were placed in an oven at 60°C for 24 h and weighed again. A factor 'F' was introduced to correct for feed losses due to water movement, aeration, siphoning and rinsing during the time that the feed was in the water. To determine this factor, 10 aquaria without shrimp were used where a known amount of feed

Table 2 Ingredient levels and proximate composition (g kg^{-1}) of the diets (AQ0 = basal diet; A = amaranth; Q = quinoa; 0–45 represent the protein level from fish meal replaced by protein from either A or Q)

Ingredients	AQ0	A15	A25	A35	A45	Q15	Q25	Q35	Q45
Fish meal*	315.6	268.3	236.7	205.2	173.6	268.3	236.7	205.2	173.6
Amaranth†	0.0	184.5	307.5	430.4	553.4	0.0	0.0	0.0	0.0
Quinoa‡	0.0	0.0	0.0	0.0	0.0	208.5	347.4	486.4	625.4
Squid meal§	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Wheat gluten¶	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Fish oil	34.8	28.4	24.2	20.0	15.8	23.5	16.0	08.4	0.9
Soybean lecithin	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cholesterol	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamins premix**	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Minerals premix††	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Carboxymethyl cellulose	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Antioxidant	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mould inhibitor	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Corn starch‡‡	450.9	320.1	232.9	145.7	58.5	301.1	201.2	101.3	1.4
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (g kg^{-1} as dry matter)									
Moisture (as was at mixing)	58.2	68.0	66.3	110.9	109.1	59.9	60.2	80.3	114.2
Crude protein ($N \times 6.25$)	308.9	301.7	293.1	289.3	288.3	308.7	303.7	302.7	302.9
Crude fat	62.4	71.6	69.0	71.0	68.3	76.4	66.7	62.5	62.9
Carbohydrate§§	499.1	487.3	501.2	462.2	467.4	483.0	500.5	488.5	455.7
Ash	71.4	71.4	70.4	66.6	66.9	72.0	68.9	66.0	64.3
Energy (MJ kg^{-1} dry matter)¶¶	168.0	168.0	167.0	1610	160.0	170.0	168.0	165.0	160.0

*Produced by steam dry method (Polar, Salango, Ecuador).

†INIAP Alegría variety, provided by Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Quito, Ecuador.

‡INIAP Tunkahuan variety, provide by INIAP, Quito, Ecuador.

§Processed in the laboratory by lyophilized from commercial frozen baby squid *Loligo* sp (71.2% crude protein c.p.; 7.5% lipid).

¶Purchase from Sigma Chemical, St. Louis, MO, USA (88.6% c.p.; 0.6% lipid).

**(mg 100 g^{-1} diet): p-amino benzoic acid, 10; thiamin-HCl, 12; riboflavin, 20; pyridoxine-HCl, 12; choline chloride, 250; nicotinic acid, 75; calcium pantothenate, 50; inositol, 200; biotin, 0.5; folic acid, 1.5; ascorbic acid, 10; menadione, 4; α-tocopherol acetate, 40; cyanocolabamine, 0.03; cholecalciferol, 0.03; β-carotene, 1.15×10^{-3}.

††(mg 100 g^{-1} diet): calcium phosphate monobasic, 272; calcium lactate, 640.2; ferric citrate, 60; magnesium sulphate heptahydrate, 274; potassium phosphate, 480; sodium phosphate monobasic, 174; sodium chloride, 86; aluminium chloride, 0.4; potassium iodide, 0.3; cuprous chloride, 0.2; manganese sulphate monohydrate, 1.6; cobalt chloride hexahydrate, 2.1; zinc sulphate heptahydrate, 7.1; sodium selenite, 2.

‡‡Purchase from Sumesa S.A., Guayaquil, Ecuador.

§§Calculated value: carbohydrate = total − (ash+crude protein+moisture+total lipid).

¶¶Total energy was calculated using following factors: 23 kJ g^{-1} protein; 35 kJ g^{-1} lipid; 15 kJ g^{-1} carbohydrate.

was placed. The amount of feed consumed (dry matter) by shrimp was expressed as a percentage of the biomass of shrimp in the aquaria and used as an indicator of diet palatability by using the expressions:

$$\text{Palatability} = \frac{\text{Supplied feed} - (\text{Non consumed} \times \text{F})}{\text{Biomass in aquarium}} \times 100$$

$$\text{Correction Factor (F)} = \frac{\text{Supplied feed}}{\text{Retrieved feed}}$$

Pellet water stability

For water stability of the diets, 2 g of pellets were weighed and placed in glass bottles (bottom area: 28.27 cm^2) with 100 mL of seawater (35 g L^{-1}). Samples in triplicate were placed in an EYELA horizontal shaker (EYELA, Model NTS-1300; Tokyo, Japan) at 70 rpm and maintained in a water bath at 28°C. After 2 h of immersion, the pellets were collected in baskets with mesh size of 600 μm, previously weighed and labelled. Meshes with the pellet were dried at 60°C for 24 h in an ISUZU

oven (Model 2-2132; Tokyo, Japan), and after this period the weight recorded.

The retention rate of dry matter (PWS) was calculated using the formula:

$$\mathrm{PWS} = 100 - (\mathrm{W\ dry}_{\text{before inmersion}} - \mathrm{W\ dry}_{\text{after inmersion}}) \times 100$$

Digestibility trial/evaluation of digestibility

At the end of the growth trial, shrimps were fed experimental diets containing 5 g kg^{-1} of chrome oxide. This compound is used as a reference component in feeds to determine relative digestibility of dry matter and protein. Chromic oxide is physiologically inert, non-toxic and can be easily mixed in formulated feeds. After a 7-day period of acclimation to the diets, faeces were collected 2 h after each feeding and grouped by aquarium. Faecal material was gently rinsed with distilled water and stored in Eppendorf tubes at −80°C. The faeces collected for 10 days were centrifuged, lyophilized and stored at −20°C. Before analysis, dried faeces were ground to a fine and homogeneous powder in Eppendorf tubes with a metallic piston. Afterwards, the faeces powder was oven dried for 24 h at 60°C and kept in a dry atmosphere using silica gel to ensure complete dryness at the time of weighing. Protein and chromic oxide contents of the diets and faeces were determined using a block digester after acid digestion (McGinnis & Kasting 1964; Foster & Gabbot 1971).

Correction Ricque-Marie, Nieto-López, Tapia-Salazar, Guajardo-Barbosa, Villareal-Cavazos, Peña-Rodriguez and Cruz-Suárez (2008) was applied for calculations of AD of protein and dry matter, using the following formula:

$$\mathrm{AD} = \left[1 - \frac{(\%\ \mathrm{Nutrient}/\%\ \mathrm{Cr_2O_3})\,\mathrm{faces}}{(\%\ \mathrm{Nutrient}/\%\ \mathrm{Cr_2O_3})\,\mathrm{nutrient}} \times \frac{1}{(1 - \%\ \mathrm{Losses}/100)}\right] \times 100$$

Growth trial at the laboratory

Juveniles of *L. vannamei* (average weight 0.5 g), originating from captive broodstock, were obtained from facilities of Centro Nacional de Acuacultura e Investigaciones Marinas (CENAIM) located in San Pedro de Manglaralto, Province of Santa Elena, Ecuador. Seven shrimps were initially stocked (39 m^{-2}) in each of the 50 L polyethylene aquaria (60 cm length × 30 cm width × 36 cm height and bottom area 0.18 m^2, which were covered with 2 mm mesh netting to prevent the shrimps jumping out) with seawater, where they were acclimatized to the experimental conditions for a week and control diet prior to onset of the experiment. After this period of adaptation, shrimp were weighed and each shrimp lost was replaced by one of similar weight.

After the adaptation period, the growth trial started with shrimp having initial average weight of 1.30 ± 0.06 g, which were fed *ad libitum* twice a day (12:00 and 18:00 hours) for 8 weeks. Faeces, moults and excess feed were siphoned from aquariums before the first feeding. The shrimp in each aquarium were weighed biweekly and at the end of the trial. Mortalities were recorded during the study period.

Water exchange in each aquarium was 300% daily, with full-strength seawater (filtered and UV-treated), using a flow-through water system. A handheld oxygen metre WTW OXY3150i (Weilheim, Germany) was used to monitor the temperature and dissolved oxygen concentration of water in each aquarium daily, and a refractometer was used to track salinity twice a week. The photoperiod was set at 12 h light: 12 h darkness.

Performance parameters

The growth parameter used to evaluate the quality of diets was calculated by the following equation:

$$\text{Specific growth rate}\,(\mathrm{SGR};\%\ \mathrm{day}^{-1}) = \left[\frac{\ln W_\mathrm{f} - \ln W_\mathrm{i}}{t(\mathrm{days})}\right] \times 100$$

Daily feed intake (DFI, % day^{-1}) was calculated with corrected feed ingestion using the expression:

$$\mathrm{DFI} = \frac{\text{Integrated feed/Averaged biomass}}{t\,(\mathrm{days})} \times 100$$

$$\text{Weight gain}\ (\%) = [(B_\mathrm{f} - B_\mathrm{i})/B_\mathrm{i}] \times 100$$

The feed conversion ratio (FCR) was estimated using the formula presented by Brand and Colvin (1977) for correcting dead shrimps:

$$\mathrm{FCR} = \frac{\text{Ingested feed}}{B_\mathrm{f} + \left[\sum \frac{W_\mathrm{i}+W_\mathrm{f}}{2} \times N\right] - B_\mathrm{i}}$$

where B_f, final biomass; B_i, initial biomass; W_i, average initial weight in each period; W_f, average final weight in each period; N, number of dead shrimp in each period.

Protein efficiency ratio (PER)
= wet weight gain (g)/protein consumed (g)

Analytical methods

Feed ingredients and diets were milled to fine powder (300 μm) and their proximate compositions were analyzed using standard laboratory procedures (AOAC 1990). Dry matter was calculated from weight loss after drying in an oven at 105°C for 2 h. Crude protein (Nx6.25) was measured using Kjeldahl method after acid digestion. Crude fat was calculated after extraction with diethyl ether extraction (Soxhlet technique). Ash was determined after ignition of the samples at 550°C for 4 h in a muffle furnace. Amino acids were determined by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) after hydrolysis of samples in 6 N HCl for 24 h at 110°C. Then, samples were derivatized with o-phthaldialdehyde according to Antoine, Wei, Littell and Marshall (1999).

Statistical analysis

All data are presented as the mean values (and SEM). The Anderson–Darling test was used to check for normality. Bartlett's test for homoscedasticity of variance was employed with $P < 0.05$ (Zar 1999). One-way ANOVA considering initial weight as covariate and, when pertinent, *a posteriori* Student–Newman–Keuls multiple comparison test were used to determine significant difference between treatments at a confidence level of 95%. The STATGRAPHICS statistical software package (Statistical Graphics System, Version Centurion, Herndon, VA, USA) was used.

Results

Water quality

Juveniles were reared in a system with a high seawater exchange rate of 300% day^{-1}. Dissolved oxygen, pH, temperature and salinity averaged 5.43 ± 0.63 mg L^{-1}, 7.88 ± 0.08, 25.5 ±1.33°C and 35 g L^{-1} respectively.

Stability and palatability of experimental diet

The pellet water stability test shows (Fig. 1) that the diet with the highest amaranth inclusion level, A45, presented the highest ($P < 0.05$) dry matter loss (33.4%). The rest of the diets containing amaranth (A15-A35) had similar values of stability to control diet, between 72.9% and 73.1%. After a 2-h immersion, the effect of quinoa content was more clearly established. Pellet stability progressively decreased as the quinoa content increased. Dry matter loss reached 52.3%, where quinoa meal was included at a rate of 45% substitution (Q45). CD presented the highest stability.

The palatability results in the laboratory trial revealed that all shrimp readily accepted the experimental diets and fed actively during this study, as no differences in palatability were obtained, ranging between 4.3 and 5.3% day^{-1}.

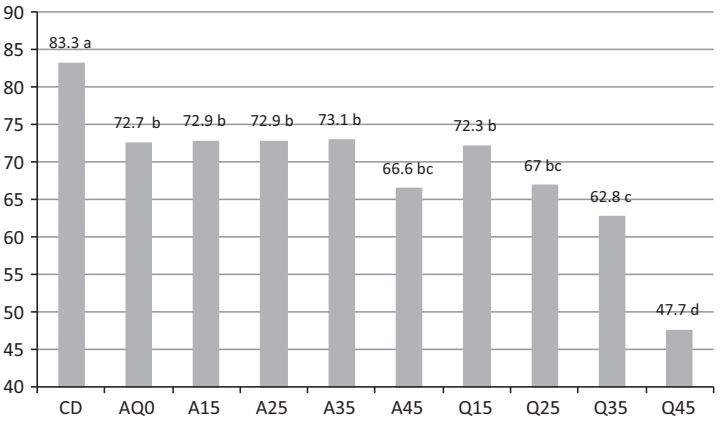

Figure 1 Dry matter pellet water stability (%) of diets containing different replacements levels of fishmeal by Amaranth and Quinoa (values with different superscripts are significantly different, $P < 0.05$).

Apparent digestibility of protein (APD) and dry matter digestibility (ADMD)

Apparent digestibility coefficient, for both dry matter and protein, presented statistical differences (Fig. 2). Amaranth diet A15 had the highest values, but A35 and A45 presented significantly ($P < 0.05$) lowest ADMD and APD. The ADMD and ADP in Quinoa diets were higher in Q15 and Q25 than control diet and Q45.

Meanwhile, both ADP and ADMD were statistically higher in diets with quinoa, 82.2% and 70.3%, than amaranth diets, 68.5% and 52.6% respectively.

Survival, growth and feed performance

Few shrimps died during the 8-week feeding trial; survival rate was not lower than 74% in diet A45, but no differences among treatments appeared (Tables 3 and 4).

The one-way ANOVA revealed that the protein source exhibited an effect on growth (final weight, total weight gain and SGR) and PER in amaranth diets (Tables 3). SGR decreased related to inclusion amaranth in diets, from 3.07 in control diet AQ0 to 2.36% day^{-1} in diet A45, and also PER, from 1.31 to 0.72 respectively. No differences were found for daily feeding intake (DFI) and conversion ratio (FCR) related to the levels of ingredients.

No differences in growth appeared in quinoa diets, but a significant effect on DFI, FCR and PER was noted (Table 4), as diet Q45 showed the lowest value of these parameters compared to the other diets.

Nevertheless, average SGR in quinoa diets was higher than in amaranth diets, 3.02 vs. 2.59; DFI was lower, 4.52 vs. 5.47; FCR was lower, 1.95 vs. 2.85; and PER higher, 1.54 vs. 0.96 respectively. Likewise, white shrimp fed most experimental diets had a higher SGR compared CD, 2.50% day^{-1} (data not shown).

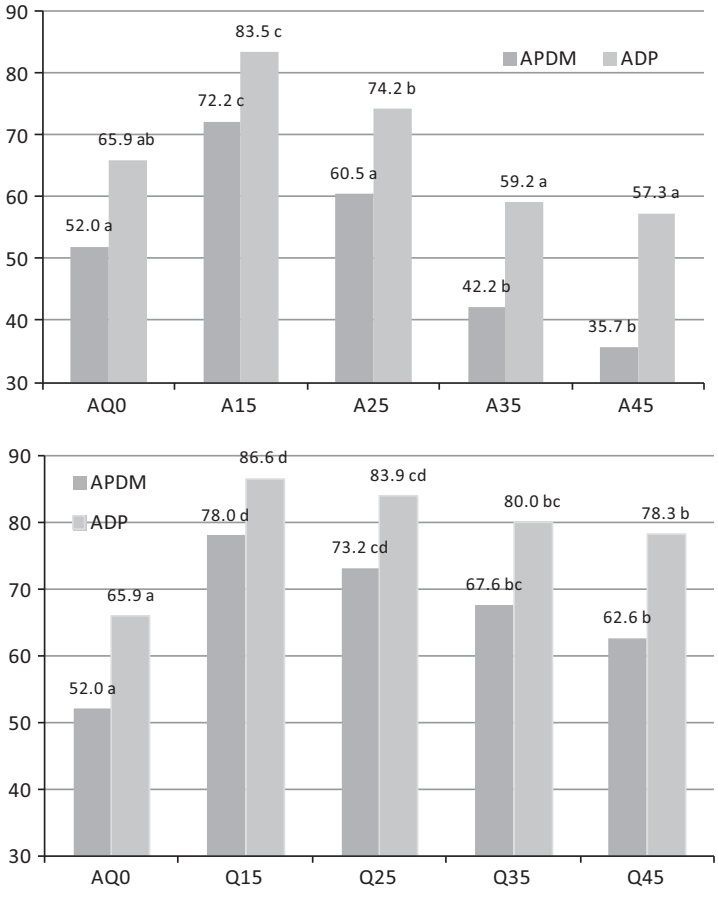

Figure 2 Apparent dry matter (ADMD) and protein (APD) digestibility (%) of experimental diets containing different replacements levels of fishmeal by Amaranth and Quinoa (values with different superscripts are significantly different, $P < 0.05$).

Table 3 Growth performance of white shrimp, *L. vannamei* reared for 8 weeks in 50-L indoor aquaria and fed the experimental diets containing different replacements levels of fishmeal by amaranth (*Amaranthus caudatus*)

Diet	AQ0	A15	A25	A35	A45	SEM
Initial weight (g shrimp^{-1})	1.34	1.31	1.30	1.32	1.31	0.02
Final weight (g shrimp^{-1})	8.33^{a}	7.17^{b}	6.59^{bc}	5.45^{c}	5.41^{c}	0.39
SGR (% day^{-1})	3.07^{a}	2.81^{a}	2.68^{ab}	2.33^{b}	2.36^{b}	0.11
Survival (%)	94.3	93.1	91.0	83.4	74.4	7.38
Total weight gain (%)	538^{a}	446^{b}	402^{bc}	316^{c}	312^{c}	30.5
DFI (%BW day^{-1})	5.40	5.62	5.87	5.65	4.76	0.70
FCR	2.29	2.57	2.77	3.28	2.91	0.24
PER	1.31^{a}	1.20^{ab}	1.08^{abc}	0.84^{bc}	0.72^{c}	0.11

Mean of six replicates using initial weight as covariate.
Values in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4 Growth performance of white shrimp, *L. vannamei* reared for 8 weeks in 50-L indoor aquaria and fed the experimental diets containing different replacements levels of fishmeal by quinoa (*Chenopodium quinoa*)

Diet	AQ0	Q15	Q25	Q35	Q45	SEM
Initial weight (g shrimp^{-1})	1.34^{a}	1.2^{b}	1.30^{ab}	1.32^{ab}	1.28^{ab}	0.02
Final weight (g shrimp^{-1})	8.31	8.68	8.16	7.86	7.33	0.44
SGR (% day^{-1})	3.10	3.16	3.06	3.00	2.89	0.08
Survival (%)	92.4	90.5	94.3	88.8	93.4	4.48
Total weight gain (%)	551	561	534	512	469	31.2
DFI (%BW day^{-1})	5.39^{a}	4.94^{a}	4.96^{a}	4.57^{a}	3.66^{b}	0.27
FCR	2.29^{a}	2.09^{a}	2.10^{a}	2.01^{a}	1.61^{b}	0.12
PER	1.28^{a}	1.40^{a}	1.47^{a}	1.42^{a}	1.87^{b}	0.09

Mean of six replicates using initial weight as covariate.
Values in the same row with different superscripts are significantly different ($P < 0.05$).

Discussion

Results of the bromatological analysis for amaranth and quinoa seed meals were similar to the results reported by Virk and Saxena (2003) and Koziol (1992) respectively. Amaranth and quinoa have a protein content (Table 1) that is comparable to alfalfa (173 g kg^{-1}) but higher than the values reported for oats (118 g kg^{-1}), sorghum (111 g kg^{-1}) and wheat (122 g kg^{-1}) (NRC 2011). In pseudo cereals, such as quinoa, albumins and globulins are the major protein fraction (440–770 g kg^{-1} of total protein), which is greater than that of prolamins (5–7 g kg^{-1}) (Koziol 1992). Crude fibre content of both meals was low when compared to the whole grain and other cereals (Koziol 1992).

Ash content in feeds decreased proportionally along with increases in the rate of inclusion of amaranth and quinoa, an effect that can be explained by the greater ash content of fishmeal compared to these meals (Table 1). The energy contained in the diets was 160–170 MJ kg^{-1}, which is similar to the reported standards for shrimp feeds (between 87 and 180 MJ kg^{-1} with an average of 159 MJ kg^{-1}) (Siccardi 2006).

Both amaranth and quinoa have high levels of certain EAA (Table 1) in comparison with fish meal, so the EAA profile of experimental diets (Table 5) was very similar to control diet. Optimum levels of several EAA have been reported for penaeid shrimp by Akiyama, Dominy and Lawrence (1992), and particularly for *L. vannamei* by Fox, Lawrence and Li-Chan (1995), Fox, Davis and Lawrence (2011), Zhou, Zeng, Wang, Wang, Wang and Xie (2012), Xie, Zeng, Zhou, Wang, Wang, Zheng and Wang (2012) and Zhou, Wang, Wang and Tan (2013). These authors reported optimum dietary levels of some EAA, 23 g kg^{-1} Arginine, 20 g kg^{-1} Lysine, 4 g kg^{-1} Methionine and 15 g kg^{-1} Threonine, which are apparently covered by experimental diets (Table 5). However, a dietary methionine requirement of 25 g kg^{-1} or 37 g kg^{-1} protein was

Table 5 Analyzed essential amino acid profile of the experimental diets expressed as g kg^{-1} protein and g kg^{-1} diet between brackets

	AQ-00	A-15	A-25	A-35	A-45	Q-15	Q-25	Q-35	Q-45
Arginine	60.5 (18.7)	66.1 (19.9)	69.9 (20.5)	73.5 (21.3)	77.3 (22.3)	62.9 (19.4)	64.5 (19.6)	66.2 (20.0)	67.8 (20.5)
Phenylalanine	41.5 (12.8)	43.2 (13.0)	44.1 (12.9)	45.4 (13.1)	47.1 (13.6)	43.7 (13.5)	45.3 (13.8)	46.8 (14.2)	48.4 (14.7)
Histidine	27.1 (8.4)	31.7 (9.6)	35.0 (10.3)	37.9 (11.0)	41.3 (11.9)	29.6 (9.1)	31.4 (9.5)	33.3 (10.1)	35.1 (10.5)
Isoleucine	44.5 (13.7)	44.2 (13.3)	44.1 (12.9)	44.1 (12.8)	43.7 (12.6)	44.8 (13.8)	44.9 (13.6)	44.8 (13.6)	45.0 (13.6)
Leucine	77.6 (24.0)	75.2 (22.7)	73.7 (21.6)	72.1 (20.9)	70.5 (20.3)	76.0 (23.5)	75.4 (22.9)	74.3 (22.5)	73.6 (22.3)
Lysine	75.2 (23.2)	72.5 (21.9)	70.6 (20.7)	68.7 (19.9)	67.0 (19.3)	72.3 (22.3)	70.3 (21.4)	68.2 (20.6)	66.5 (20.1)
Methionine	29.4 (9.1)	27.7 (8.4)	26.1 (7.6)	24.9 (7.2)	23.7 (6.8)	29.3 (9.0)	29.1 (8.8)	29.2 (8.8)	29.0 (8.8)
Threonine	42.8 (13.2)	46.2 (13.9)	48.6 (14.2)	50.9 (14.7)	53.3 (15.4)	44.1 (13.6)	44.9 (13.6)	45.8 (13.9)	46.7 (14.1)
Tryptophan	11.4 (3.5)	10.1 (3.0)	9.2 (2.7)	8.2 (2.4)	7.6 (2.2)	10.1 (3.1)	9.1 (2.8)	8.1 (2.5)	7.5 (2.3)
Valine	49.8 (15.4)	48.2 (14.5)	46.9 (13.7)	45.8 (13.2)	44.7 (12.9)	48.5 (15.0)	47.7 (14.5)	46.5 (14.1)	45.7 (13.8)
Sum of EAA	459.7	465.2	468.3	471.5	476.1	461.3	462.7	463.2	465.2

recommended, respectively, by Akiyama *et al.* (1992) and Fox, Lawrence, Patnaik, Forster, Ju and Dominy (2010) for shrimp feeds; so a potential deficiency in methionine may be responsible for the depressed growth of shrimp fed diets with higher levels of fish meal replaced by amaranth A-35 and A-45 if protein digestibility is taken into account (Fig. 2), because the availability of methionine is more likely to be reduced, as the situation worsens as fishmeal is replaced by amaranth in these diets. It is therefore reasonable to say that including amaranth would also have a negative effect on weight gain due to a lower availability of methionine. Thus, the optimal supplementation of methionine must be considered in future studies on this species. Likewise, optimum requirements of Methionine, and other EAA, must be studied in *L. vannamei*, because the two cited available references (4 and 25 g kg^{-1}) are very different, even considering other shrimp species (NRC 2011), in the interval of 7–9 g kg^{-1}, respectively, for *Penaeus monodon* and *Marsupenaus japonicus*, whereas optimum levels of Lysine, for example, are very similar, 19–21 g kg^{-1} for three species.

The data on pellet water stability showed that percent recovery of experimental diets was lower than CDs due to the different manual and industrial preparation processes respectively. Amaranth diet stabilities were similar to those of control diet AQ0, with the exception of the highest replacement (A45), and they were generally high (>72%) after a 2 h period of submersion in seawater (Fig. 1). On the other hand, lower levels of pellet water stability were observed in diets replaced with 250, 350 and 450 g kg^{-1} fish meal protein by quinoa protein, which may be attributed to its low amylose level, and firstly to the lower content of dietary corn starch, because although the starch granule has high swelling capacity, structural bonding is weak and the starch cannot withstand the stresses caused by swelling and water movement in diets with corn starch level lower than 150 g kg^{-1}, A45, Q35 and Q45. Water stability of feeds containing amaranth and quinoa found in this study was lower when compared with results reported by Molina-Poveda *et al.* (2013, 2014), 82–95% in lupin diets and 82–87% using corn gluten meal, all of which had corn starch levels up 300 g kg^{-1}. The relatively poor stability of diets could affect their nutritional values of vitamins or amino acids but Fox *et al.* (2010) reported

a minimal leaching of methionine, and quinoa diets gave a good growth in comparison to CD, which presented a higher stability.

In this study, palatability was not affected by the inclusion of quinoa in any of the fish meal replacement levels, but feed intake of diet Q45 was the lowest, which would show that the extraction of saponins in quinoa was not enough and the long-term ingestion was affected with an inclusion of 550 g kg^{-1}.

On the other hand, it has been reported that amaranth reduced feed intake, likely as a consequence of antinutritional factors accumulation (Agbaire 2011; Montero-Quintero, Molina & Sánchez-Urdaneta 2011), or tannins causing palatability problems due to the astringent taste (Joslyn & Goldstein 1964), although no difference was observed in the current trial. Becker, Wheeler, Lorenz, Stafford, Grosjean, Betschart and Saunders (1981) evaluated 10 different samples of amaranth and found a range of 0.08–0.42 g kg^{-1} of tannins. For example, it is known that carp are very sensitive to adverse taste caused by these factors (Becker & Makkar 1999). Tannin levels in the seed coat of amaranth are higher than those in the perisperm and dark-seeded varieties are known to have higher tannin levels than non-dark-seeded varieties (Lorenz & Wright 1984). Bressani, Elias and Garcia-Soto (1989) found an improvement in mean weight gain and in protein quality for the cooked grains, contrary to reported before by Pedersen, Hallgren *et al.* (1987). In the present work, grain amaranth of light-coloured seeds was used and amaranth was not processed, and although daily feed intake was similar to quinoa diets and control diet, the growth was lower, which could indicate the negative effect of antinutritional factors.

Quinoa meal, even at the highest level of protein replacement (450 g kg^{-1}) in diets, can be effectively utilized by the shrimp, as evidenced by the non-significant differences in growth rate of shrimp from 0 to highest level of quinoa meal feed replacement in the diet, and the best feed conversion and protein efficiency of diet Q45. It seems clear that the EAA composition was well balanced in the diet and the levels of antinutritional factors were below the levels that might inhibit growth in *L. vannamei*.

On the contrary, amaranth meal, even at 150 g kg^{-1} substitution, gave a lower final weight, which agrees with the work of Adeniji, Fakoya and Omamohwo (2007) who fed fingerlings of tilapia (*Oreochromis niloticus*) with diets containing 50–750 g kg^{-1} *A. spinuous* and reported reduced growth of fish at all levels of inclusion. Other studies on the use of *A. spinuous* leaf meals as dietary protein source have been conducted for tilapia and catfish, with variable results (Adeniji *et al.* 2007; Adewolu & Adamson 2011). Adewolu and Adamson (2011) also reported that up to 50 g kg^{-1} of *A. spinosus* leaf meal can be included in a practical diet for African catfish *C. gariepinus*, and in general there was a decreasing trend in growth rate of fingerlings with increasing inclusion level from 100 to 200 g kg^{-1} in experimental diets. These results, which keep a similar pattern to this study, might be due to presence in amaranth of saponins, alkaloids, tannins, phytates and oxalates as antinutritional substances (Lorenz & Wright 1984; Agbaire 2011; Montero-Quintero *et al.* 2011). A serine proteinase inactivating proteins has also been isolated from seeds of grain *Amaranthus caudatus* L. (Hejgaard, Dam, Petersen & Bjørn 1994). Although this inhibitor of trypsin is very heat, stable and is immediately inactivated by pepsin at pH 2 (Hejgaard *et al.* 1994), this inhibitor represents a potential antinutritional factor in feeds for shrimp that lack pepsin and whose digestive pH does not reach 2, but optimum pH for digestive enzymes in shrimp gut range between 4.5 and 9.5 (Lan & Pan 1990; Souza, Fernandes, Silva, Lemos, Bezerra & Souza 2009).

The decreasing level of digestibility of the diets in the shrimp with an increase in the level of amaranth meal replacement may result from the presence of serine proteinase in this ingredient, which reaches high levels in diets A45 and A35. Lower digestibility of the amaranth diets 25, 35 and 45 in shrimp, as shown in the digestibility coefficients for dry matter and protein obtained in this study, may be another contributing factor in the poor growth of shrimp fed diets with various levels of amaranth meal replacement. On the other hand, even at the highest level of fishmeal replacement, quinoa meal is still highly digestible and the ADMD (78–63%) and APD (87–78%) values obtained in this study are comparable to the results of Gutiérrez-Espinosa *et al.* (2011) and similar to those for other cereals, but these authors reported digestibility coefficients of quinoa meal in tilapia nilotica of 58.8–64.4% for ADMD and 67.7–77.5% for APD in tiger shrimp.

This study reported a high PER and a relatively high AD for washed quinoa compared to amaranth (70 vs. 53% for ADMD and 82 vs. 68% for APD). Based on PER, protein digestibility and nitrogen balance Ranhotra, Gelroth, Glaser, Lorenz and Johnson (1993) found that the quality of protein in quinoa equals that of casein. On the other hand, Pedersen, Kalinowski and Eggum (1987) using chemical score suggested leucine, valine or threonine as the limiting amino acids in amaranth grain. Finally, considering the growth and feed conversion results, it seems that quinoa is a better ingredient than amaranth for substituting fish meal in white shrimp diets.

Conclusion

This study has demonstrated the acceptable nutritional value of quinoa meal as ingredient for shrimp diets, as this raw material can replace up to 45% of fish meal in feeds for *L. vannamei*. This species shows no adverse effect on growth, survival, feed palatability, intake and digestibility when fed with diets having up to 620 g kg^{-1} of this ingredient. On the other hand, the amaranth meal was not an effective as an ingredient, but the exact causes for the growth-depressing effects of raw grain amaranth are unclear. Further studies are necessary to investigate whether processing amaranth by cooking and/or addition of limiting amino acids in diets should improve shrimp performance. In addition, it would be interesting to establish whether the use of quinoa protein concentrate can completely replace fish meal in diets for shrimps.

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