Aquaculture Nutrition

Aquaculture Nutrition 2017 **23**; 293–303 doi: 10.1111/anu.12392

Effects of soy protein ratio, lipid content and minimum level of krill meal in plant-based diets over the growth and digestibility of the white shrimp, *Litopenaeus vannamei*

H. SABRY-NETO[1], D. LEMOS[2], T. RAGGI[2] & A.J.P. NUNES[1]

[1] *LABOMAR – Instituto de Ciências do Mar, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil;* [2] *Laboratório de Aquicultura (LAM), Instituto Oceanográfico, Universidade de São Paulo (USP), São Paulo and Ubatuba, São Paulo, Brazil*

Abstract

This study evaluated the effects of soy protein ratio, lipid content and the minimum dietary level of krill meal in plant-based diets over the growth performance and digestibility of *Litopenaeus vannamei*. Nine plant-based diets varied the soybean meal (SBM) and soy protein concentrate (SPC) inclusion ratio at 1 : 2.3, 1 : 1 and 2.5 : 1, and their dietary lipid content at 121.4 ± 9.4, 102.3 ± 1.2, and 79.9 ± 1.2 g kg^{-1} (in a dry matter basis). An additional diet containing 120 g kg^{-1} of fish meal (salmon by-product) was used as a control. Krill meal was included at 0, 5, 10, 20 and 30 g kg^{-1} in a new set of plant-based diets. After 10 weeks in clear-water tanks of 0.5 m^3, no effect of SBM:SPC ratio and dietary lipid content was detected on shrimp survival. However, dietary lipid levels of 80 and 121 g kg^{-1} combined with a high SPC to SBM resulted in the lowest final body weight and the poorest apparent crude protein digestibility, respectively. Krill meal increased feed intake at only 10 g kg^{-1}, while at 20 g kg^{-1}, it accelerated shrimp growth, increased yield and reduced food conversion ratio.

KEY WORDS: digestibility, krill meal, performance, plant-based diets, shrimp, soy proteins

Received 12 January 2015; accepted 29 August 2015

Correspondence: A.J.P. Nunes, LABOMAR – Instituto de Ciências do Mar, Universidade Federal do Ceará, Avenida da Abolição, 3207 – Meireles, Fortaleza, Ceará 60.165-081, Brazil. E-mail: alberto.nunes@ufc.br

Introduction

The ability of cultured penaeids to process and absorb nutrients from a wide variety of plant feedstuffs has been demonstrated in several studies. Partial or complete replacement of fish meal in shrimp diets has been achieved with single sources of plant proteins, including soybean meal (SBM; Alvarez *et al.* 2007; Bulbul *et al.* 2014), soy protein concentrate (SPC; Paripatananont *et al.* 2001; Sookying & Davis 2009; Bauer *et al.* 2012; Sá *et al.* 2013), rice protein concentrate (Oujifard *et al.* 2015), lupin meal (Molina-Poveda *et al.* 2013) and canola meal (Cruz-Suárez *et al.* 2001; Bulbul *et al.* 2014), under various culture systems and rearing conditions.

Studies have shown that the Pacific white shrimp, *Litopenaeus vannamei*, can be successfully raised without deleterious growth effects with plant-based diets. Dietary protein in these diets have originated primarily from terrestrial plant feedstuffs, mainly SBM (Roy *et al.* 2009; Fox *et al.* 2011; Morris *et al.* 2011; Sookying & Davis 2011; Sookying *et al.* 2011, 2013). This has been possible due to a better understanding of shrimp nutrient requirements (NRC 2011) and the commercial availability of more refined sources of soy proteins (Cruz-Suárez *et al.* 2009; Sá *et al.* 2013) and crystalline amino acids (Forster & Dominy 2006; Fox *et al.* 2011; Browdy *et al.* 2012; Gu *et al.* 2013; Nunes *et al.* 2014; Bulbul *et al.* 2015). These developments have partially overcome the antinutritional factors (Francis *et al.* 2001) and nutrient deficiency characteristic of many plant feedstuffs (Gatlin *et al.* 2007).

The increased use of plant proteins in shrimp diets require a more comprehensive approach that will lead to a wider application of the plant-based diet concept and a reduced reliance on marine proteins. This includes aspects related to ingredient and nutrient balance, digestibility and rational use of marine proteins. This study aimed at evaluating the effects of soy protein ratio, lipid content and the minimum allowable dietary level of krill meal in plant-based diets to enhance the growth performance and digestibility for juveniles of the white shrimp, *L. vannamei*.

Materials and methods

Experimental design

This study was divided into three phases carried out consecutively with juveniles of the white shrimp, *L. vannamei*, to determine the following: (1) the effect of plant-based diets in the growth performance of shrimp raised in clear water; (2) the apparent digestibility coefficients (ADC) for crude protein, crude lipid and gross energy of plant-based diets for juvenile shrimp; and (3) the minimum allowable dietary level of krill meal in plant-based diets to enhance shrimp growth.

For phases (1) and (2), nine diets were formulated to contain over 900 g kg^{-1} of their composition or all of their protein content derived from plant ingredients of terrestrial or aquatic origin. Diets were split into three sets, each varying the dietary inclusion ratio of soybean meal and soy protein concentrate (SBM : SPC ratio) and their lipid content. An additional diet was used as a control, containing 450.0, 120.0 and 19.5 g kg^{-1} (as is basis) of SBM, fish meal (salmon by-product), and meat and bone meal, respectively. In phase (3), a basal plant-based diet was formulated to contain no krill meal. From this diet, four other diets were prepared by gradually increasing the dietary levels of krill meal at 5, 10, 20 and 30 g kg^{-1} (as is basis).

Study site, rearing systems and management

Studies on shrimp growth performance were carried out at LABOMAR's marine aquaculture experimental station (State of Ceará, Brazil). In phase (1), shrimp of 3.13 ± 0.35 g (mean ± standard deviation; n = 2000) were stocked at 72 animals m^{-2} (41 shrimp per tank) in 50 tanks of 0.5 m^3. The indoor tank system operated under a clear-water condition throughout the complete rearing cycle. The system is organized in rolls of five individual tanks, totalling two separate sets of 50 rearing tanks. In each set, sea water flows in and out individually to each rearing tank served by a single sand and cartridge filtering system connected to a three-stage storage tanks with a 40 m^3 total capacity. The filtering system operates to remove any particle larger than 50 µm, which may act as a potential food source to stocked shrimp. In all rearing tanks, there is continuous aeration provided by two 2.0-hp air blowers and two air diffusers per tank. All incoming sea water was filtered prior to use and disinfected with sodium hypochlorite at 5 ppm (65% of active chlorine; HTH®, Nordesclor S.A., Igarassu, Brazil). A 55-kvA (Kilo Volt Amperes or 44 kW) diesel generator was used as a back-up power supply in case of power failure.

Shrimp were acclimated for 13 days with a 350 g kg^{-1} crude protein marine shrimp feed. Starting at 3.97 ± 0.52 g (n = 500) body weight, shrimp were fed on nine plant-based and one control diet for 73 days. Five replicate tanks were used for each diet type. Over the course of the rearing period, water salinity, pH and temperature reached 36 ± 2 g L^{-1} (n = 2950), 7.85 ± 0.18 (n = 2950), and 28.5 ± 0.56 °C (n = 2950), respectively.

In study phase (3), shrimp of 2.84 ± 0.52 g (n = 800) were stocked at 70 shrimp m^{-2} (40 shrimp per tank) and raised for 72 days with five experimental diets in 20 indoor tanks of 0.5 m^3 (four replicate tanks per dietary treatment). During the rearing period, water quality was kept at 35 ± 1 g L^{-1} (n = 1260) salinity, 7.95 ± 0.36 (n = 1260) pH and 28.2 ± 0.65 °C (n = 1260) temperature. In rearing phases (1) and (3), water quality and feed management followed a similar protocol as described by Nunes *et al.* (2011). Shrimp were fed in excess throughout the rearing period.

In phase (2), apparent digestibility (protein, lipid and energy) of plant-based diets with 5 g kg^{-1} chromic oxide inclusion was evaluated at the aquaculture facilities of University of São Paulo (Oceanographic Institute, Ubatuba, State of São Paulo, Brazil) over 50 days. Shrimp of 4.69 ± 0.17 g (n = 1350) were stocked in 30 slightly conical circular tanks (three replicates per diet in addition to control) of 0.5 m^3 each (1040 × 675 mm; diameter × height) under 70 shrimp m^{-2} (45 shrimp per tank). All rearing tanks had an individual settling device, as described by Carvalho (2011) and Carvalho *et al.* (2013), attached to Falcon tubes of 50 mL for settling and collection of shrimp faeces and feed remains. Shrimp were fed continuously by belt feeders from 12:00 a.m. to 7:00 a.m. (80% of daily ration) and at 11:30 a.m. (20% of daily ration). Feed rations were calculated based on shrimp body weight and survival. Occasional uneaten pellets were collected daily from settling tubes positioned on the tank bottom and discarded. Faeces collection took place 5 days week^{-1}, 1 h after cleaning of settling tubes, in the morning (9:15, 10:15 and 11:15 a.m.) and afternoon (1:30; 2:30 and 3:30 p.m.). Faeces samples were filtered by vacuum pumping, following gently rinsing with distilled water for salt removal and storage at −20 °C. All samples were freeze-dried prior to chemical analysis.

Diets and faeces samples were analysed in replicates for crude protein (micro Kjeldahl), total lipid (ether extract) and gross energy (adiabatic calorimetric bomb) according to AOAC (2002). The inert marker content in diets and faeces was analysed as chromium in replicates by inductively coupled plasma atomic emission spectroscopy (ICP-AES) by an Arcos-SOP (Spectro Analytical Instruments GmbH, Kleve, Germany) with standard chromium solution (1.00 ± 0.003 mg g^{-1}; Specsol, Jacareí, São Paulo, Brazil) tracked by a standard reference material 3112a – NIST – USA. ADC of test diets were calculated as: ADC (%) = 100 – [100 (% nutrient or energy in faeces/% nutrient or energy in diets) × (% marker in diets/% marker in faeces)].

Diets

Nine plant-based diets and one control were formulated to be isonitrogenous (444.2 ± 6.2 g kg^{-1}, dry matter basis) and isoenergetic (21.2 ± 0.5 MJ kg^{-1}). Plant-based diets were divided into three sets, each one varying the dietary inclusion ratio of SBM and SPC, and their dietary lipid content (Table 1). Plant ingredients were of terrestrial or aquatic origin occupying over 900 g kg^{-1} of the total diet composition. Wheat flour was used at a fixed inclusion of 200.0 g kg^{-1} (as is basis) for improved water pellet stability, while Spirulina meal was added at 5.0 g kg^{-1} as a palatability enhancer (Silva-Neto *et al.* 2012).

Soybean meal and SPC acted as the major dietary sources of protein. Their inclusion ratio (SBM : SPC) met nearly approximate ratios of 1 : 2.3 (346 and 150 g kg^{-1}, respectively), 1 : 1 (260 and 260 g kg^{-1}), and 2.5 : 1 (400 and 155 g kg^{-1}). For every set of diets with a SBM : SPC ratio, three levels of crude lipid were adopted as follows: 121.4 ± 9.4, 102.3 ± 1.2 and 79.9 ± 1.2 g kg^{-1} (in a dry matter basis). Dietary lipid changed by varying soybean oil content. In order to accommodate different levels of soybean oil and SBM, inclusion of corn gluten meal and broken rice varied slightly between diets. Salmon oil, soy lecithin and cholesterol were included at a fixed level of 20.0, 20.0, and 1.0 g kg^{-1} of the diet, to meet the minimum dietary concentrations of omega-3 polyunsaturated fatty acids (*n*-3 LC-PUFA; Sá *et al.* 2013), phospholipids and cholesterol (Gong *et al.* 2000; Morris *et al.* 2011), respectively.

An additional diet was formulated to act as a control. This diet contained nearly the same ingredients as the plant-based diets, except for salmon by-product meal and meat and bone meal included at 120.0 and 19.5 g kg^{-1}, respectively. No Spirulina meal was included in this diet, and dietary inclusion of SBM was increased to 450.0 g kg^{-1}. Dietary levels of SPC, corn gluten meal and salmon oil were reduced to 4.3, 40.3 and 10.0 g kg^{-1}, respectively. Diets used for the apparent *in vivo* digestibility assay were prepared using chromic oxide (Cr_2O_3) as the inert marker. Chromic oxide was incorporated into all formulas at 5 g kg^{-1} at the cost of wheat flour.

A new set of plant-based diets were prepared to investigate the minimum allowable dietary levels of krill meal to enhance shrimp growth (Table 2). These diets contained a SBM : SPC ratio of 1 : 2 (150 and 300 g kg^{-1}) and a crude lipid content of 109.1 g kg^{-1}. Krill meal was included at 5, 10, 20 and 30 g kg^{-1} at the cost of corn gluten meal on a protein basis. As dietary inclusion of krill meal raised, soybean oil was adjusted to maintain crude lipid consistent throughout all diets. An additional diet, without any krill meal, was used as a control.

Diets from all experiments were manufactured with laboratory equipment as described in Nunes *et al.* (2011). Dry matter (drying in a convection oven between 103 and 105 °C), ash (sample incineration in a muffle furnace at 550 °C), crude protein (Kjeldahl method of nitrogen estimation), crude lipid (resulting residue extracted with diethyl ether), crude fibre (acid and alkaline hydrolysis) and gross energy (bomb calorimetry) were determined following standard methods (AOAC 2002).

Growth performance, digestibility and statistical analyses

In all experiments, shrimp were individually weighed at stocking and harvested to determine their initial and final body weight (g), weekly growth rate (g $week^{-1}$), yield (g of shrimp gained m^{-2}) and survival (%). Food conversion ratio (FCR) was calculated based on apparent feed intake (g of feed ingested per stocked shrimp) determined at a dry matter basis according to Nunes *et al.* (2006).

The effect of SBM : SPC ratio and dietary lipid content on shrimp growth performance and feed digestibility was analysed by two-way ANOVA arranged in a 3 × 3 factorial design. The effect of dietary inclusion of krill meal on shrimp growth performance was analysed by one-way ANOVA for completely randomized experiments. The statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used. When significant differences were detected between the means, they were compared two-by-two with the Tukey's HSD test. The significant level of 5% was set in all statistical analyses.

Results

After 72 days feeding on plant-based diets, final shrimp survival exceeded 84% (Table 3). There was no statistically

Table 1 Ingredient and proximate composition of plant-based diets

Ingredients	Diets/Composition (g kg^{-1} of the diet, as is)[1]									
	1 : 2.3/121	1 : 2.3/102	1 : 2.3/80	1 : 1/121	1 : 1/102	1 : 1/80	2.5 : 1/121	2.5 : 1/102	2.5 : 1/80	Control
SPC[2]	345.0	345.0	346.7	260.0	260.0	260.0	157.5	154.6	152.2	4.3
SBM[3]	150.0	150.0	150.0	260.0	260.0	260.0	400.0	400.0	400.0	450.0
Wheat flour[4]	200.0	200.0	200.0	200.0	200.0	200.0	194.9	200.0	200.0	200.0
Corn gluten meal[5]	67.1	64.9	62.2	74.9	72.7	70.4	80.0	80.0	80.0	40.3
Soybean oil[6]	67.0	46.3	21.1	65.4	44.7	24.0	63.5	42.7	21.9	10.0
Broken rice[7]	67.0	90.0	100.0	35.7	58.7	81.7	0.0	18.6	41.8	77.5
Spirulina meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0.0
Salmon by-product meal[8]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	120.0
Meat and bone meal[9]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.5
Magnesium sulphate	6.2	6.2	7.0	6.1	6.1	6.1	6.1	6.1	6.1	6.1
Common salt	4.7	4.7	5.0	4.9	4.9	4.9	5.0	5.0	5.0	0.0
Others[10]	88.0	88.0	88.0	88.0	88.0	88.0	88.0	88.0	88.0	72.2
Proximate composition (g kg^{-1} of the diet, dry matter basis)										
Crude protein[15]	448.0	452.8	443.2	437.5	451.3	448.8	442.6	440.8	444.7	432.6
Crude lipid[15]	125.9	102.8	78.5	127.5	103.2	81.0	121.0	100.9	80.2	71.4
Crude fibre[15]	14.7	14.6	13.2	19.0	16.2	17.9	25.1	24.1	22.2	23.1
Ash[15]	125.9	102.8	78.5	127.5	103.2	81.0	121.0	100.9	80.2	71.4
Nitrogen-free extract[16]	338.2	355.3	380.8	340.1	351.5	374.6	330.2	352.9	371.6	382.6
Gross energy[15] (MJ kg^{-1})	21.9	21.5	21.1	20.7	20.4	21.0	21.5	21.4	21.4	21.1

[1] Soybean meal (SBM) : soy protein concentrate (SPC) ratio/dietary crude lipid content.
[2] Sementes Selecta S.A. (Goiânia, Brazil). 626.3 g kg^{-1} crude protein (protein); 7.7 g kg^{-1} crude lipid (lipid); 42.3 g kg^{-1} ash; 43.3 g kg^{-1} crude fibre (fibre); 82.2 g kg^{-1} moisture.
[3] Farelo de Soja 46; Bunge Alimentos S.A. (Luis Eduardo Magalhães, Brazil). 455.8 g kg^{-1} protein; 22.8 g kg^{-1} lipid; 61.5 g kg^{-1} ash; 56.7 g kg^{-1} fibre; 111.1 g kg^{-1} moisture.
[4] Dona Benta Tipo 1; J. Macedo S.A. (Fortaleza, Brazil). 119.5 g kg^{-1} protein; 30.1 g kg^{-1} lipid; 5.8 g kg^{-1} ash; 0.5 g kg^{-1} fibre; 100.0 g kg^{-1} moisture.
[5] Protenose®; Ingredion Brasil (São Paulo, Brazil). 657.9 g kg^{-1} protein; 26.7 g kg^{-1} lipid; 13.7 g kg^{-1} ash; 17.4 g kg^{-1} fibre; 75.6 g kg^{-1} moisture.
[6] Bunge Alimentos S.A. (Luis Eduardo Magalhães, Brazil). 980.0 g kg^{-1} lipid.
[7] Usina Catende (Catende, Brazil). 65.2 g kg^{-1} protein; 15.3 g kg^{-1} fat; 9.0 g kg^{-1} ash; 4.3 g kg^{-1} fibre; 116.7 g kg^{-1} moisture.
[8] Pesquera Pacific Star S.A. (Puerto Montt, Chile). 739.1 g kg^{-1} protein; 122.6 g kg^{-1} lipid; 156.7 g kg^{-1} ash; 8.56 g kg^{-1} fibre; 110.9 g kg^{-1} moisture.
[9] NORDAL Nordeste Indl. de Derivados Animais Ltda. (Maracanaú, Brazil). 409.7 g kg^{-1} CP; 151.1 g kg^{-1} lipid; 335.98 g kg^{-1} ash; 26.6 g kg^{-1} fibre; 67.8 g kg^{-1} moisture.
[10] Others (in plant-based and in control diets) included, respectively: 20.0 and 9.18 g kg^{-1} of dicalcium phosphate; 20.0 g kg^{-1} of vitamin–mineral premix[11]; 20.0 and 25.0 g kg^{-1} of soy lecithin[12]; 20 and 10 g kg^{-1} of salmon oil[13]; 10.0 g kg^{-1} of potassium chloride (only in diet 1 : 2/100); 7.0 g kg^{-1} of synthetic binder; 1.0 g kg^{-1} of cholesterol[14].
[11] Rovimix Camarão Extensivo, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). Guarantee levels per kg of product: vitamin A, 1 000 000 IU; vitamin D3, 300 000 IU; vitamin E, 15 000 IU; vitamin K3, 300.0 mg; vitamin B1, 3000 mg; vitamin B2, 2500 mg; vitamin B6; 3500 mg; vitamin B12, 6.0 mg; nicotinic acid, 10 000 mg; pantothenic acid, 5000 mg; biotin, 100.0 mg; folic acid, 800.0 mg; vitamin C, 25 000 mg; choline, 40 000 mg; inositol, 20 000 mg; iron 2000 mg; copper, 3500 mg; chelated copper, 1500 mg; zinc, 10 500 mg; chelated zinc, 4500 mg; manganese, 4000 mg; selenium, 15.0 mg; chelated selenium, 15.0 mg; iodine, 150.0 mg; cobalt, 30.0 mg; chromium 80.0 mg; filler, 1000 g.
[12] Courtesy of InVivo Nutrição e Saúde Animal Ltda. (Paulínia, Brazil). 927.6 g kg^{-1} lipid; 61.1 g kg^{-1} ash; 620.0 total phospholipids.
[13] Pesquera Pacific Star S.A. (Puerto Montt, Chile). 980.0 g kg^{-1} lipid.
[14] Cholesterol SF, 91% of active cholesterol (minimum). Dishman Netherlands B.V. (Veenendaal, The Netherlands).
[15] Analysed values.
[16] Calculated by difference (1000 – protein–lipid–fibre–ash).

significant effect of dietary SBM : SPC ratio or lipid content on final survival ($P > 0.05$, two-way ANOVA). However, shrimp fed diets containing an SBM : SPC ratio and lipid content of 1 : 1 and 121 g kg^{-1}, 2.5 : 1 and 102 g kg^{-1}, and 2.5 : 1 and 80 g kg^{-1}, respectively, achieved a lower survival compared with those fed the control diet ($P < 0.05$, Tukey's HSD). Shrimp mean weekly growth (0.72 ± 0.10 g), gained yield (532 ± 89 g m^{-2}), feed intake

Table 2 Ingredient composition of plant-based diets with minimum inclusion of krill meal

	Diets/Composition (g kg^{-1} of the diet, as is)[1]				
		Dietary inclusion of krill meal (g kg^{-1}, as is)			
Ingredients[1]	Control	5	10	20	30
Soy protein concentrate	300.0	300.0	300.0	300.0	300.0
Wheat flour	240.0	240.0	240.0	240.0	240.0
Soybean meal	150.0	150.0	150.0	150.0	150.0
Corn gluten meal	108.4	104.0	99.5	90.6	81.8
Broken rice	57.6	57.9	58.1	58.7	59.1
Soybean oil	45.1	44.3	43.5	41.8	40.2
Krill meal[2]	0.0	5.0	10.0	20.0	30.0
Common salt	4.8	4.8	4.8	4.7	4.7
Others[3]	94.1	94.1	94.1	94.1	94.1
Proximate composition (g kg^{-1} of the diet, dry matter basis)					
Crude protein[4]	392.6	392.6	392.7	392.7	392.7
Crude lipid[4]	109.1	109.1	109.1	109.1	109.1
Crude fibre[4]	26.0	26.1	26.4	26.9	27.4
Ash[4]	28.9	29.6	30.2	31.5	32.8
Nitrogen-free extract[5]	443.4	442.6	441.7	439.8	438.0
Gross energy[6] (MJ kg^{-1})	20.2	20.2	20.2	20.1	20.1

[1] For ingredient composition, refer to Table 1.
[2] QRILLTM meal, Aker Biomarine ASA (Oslo, Norway). 580.0 g kg^{-1} crude protein; 180.0 g kg^{-1} lipid; 130.0 g kg^{-1} ash; 60.0 g kg^{-1} crude fibre; 71.0 g kg^{-1} moisture.
[3] Others included: 20.0 g kg^{-1} of dicalcium phosphate; 20.0 g kg^{-1} of vitamin–mineral premix; 20.0 g kg^{-1} of soy lecithin; 20 g kg^{-1} of salmon oil; 7.0 g kg^{-1} of synthetic binder; 6.1 g kg^{-1} of magnesium sulphate; 1.0 g kg^{-1} of cholesterol.
[4] Projected values based on ingredient composition.
[5] Calculated by difference (1000 – protein–lipid–fibre–ash).
[6] Calculated using an energy value of protein, lipid and carbohydrate of 5.64, 9.44 and 4.11 kcal g^{-1}, respectively.

(16.8 ± 1.3 g stocked shrimp^{-1}) and FCR (2.34 ± 0.48) did not differ statistically among experimental diets ($P > 0.05$, one-way ANOVA).

Shrimp final body weight was also not depressed when they were fed on plant-based diets (Fig. 1). However, a decline in final body weight was detected when the lipid content in diets with an SBM : SPC ratio of 1 : 2.3 was reduced to 80 g kg^{-1} ($P < 0.05$, one-way ANOVA). Comparatively, shrimp fed this diet also achieved a lower body weight than those fed the control ($P < 0.05$, Tukey's HSD). A poor and non-significant correlation was detected between shrimp percentage weight gain and the dietary SBM : SPC ratio or their lipid content ($P > 0.05$; Pearson coefficient of correlation).

In the digestibility assay, shrimp were harvested with 9.48 ± 0.68 g body weight after 50 days of rearing. There were no statistically significant differences between dietary treatments for final shrimp survival (82.2 ± 10.4%; $P > 0.05$, one-way ANOVA). Final body weight was significantly higher in control diet (10.82 ± 2.60 g), but it did not differ compared with diet SBM : SPC at 1 : 1 ratio and 80 g kg^{-1} lipid ($P > 0.05$). Weekly growth rate was also higher with the control diet (0.85 ± 0.03 g, $P < 0.05$) except when compared to diets with 1 : 1 SBM : SPC ratio and 80 g kg^{-1} lipid, and 2.5 : 1 SBM : SPC and 102 g kg^{-1} lipid ($P > 0.05$). Likewise, FCR was significantly lower in the control treatment (1.77 ± 0.05 g) in comparison with most other diets ($P < 0.05$). However, no statistically significant difference was found in FCR between the control and diets containing 2.5 : 1 SBM : SPC either at 80 or 102 g kg^{-1} lipid, and at 1 : 1 SBM : SPC with 80 g kg^{-1} lipid ($P > 0.05$).

Apparent crude protein digestibility (ACPD) of plant-based diets ranged from 71.3 ± 1.53% to 78.5 ± 2.90% (Table 4). ACPD was significantly affected by the dietary SBM : SPC ratio ($P < 0.05$, two-way ANOVA), but not the lipid content ($P > 0.05$). At a dietary lipid content of 102 g kg^{-1}, there was a trend towards an improved ACPD when the SBM : SPC ratio shifted from 1 : 2.3 to 2.5 : 1 ($P < 0.05$, Tukey's HSD). There was no statistically significant difference in ACPD between plant-based diets and the control (82.1 ± 2.42%; $P > 0.05$, Tukey's HSD), except when compared to the diet with a SBM : SPC ratio of 2.5 : 1 and a lipid content of 121 g kg^{-1} ($P < 0.05$).

Digestibility of crude lipid in plant-based diets was significantly affected by both the SBM : SPC ratio and their lipid content ($P < 0.05$, two-way ANOVA). Although the apparent crude lipid digestibility (ACLD) exceeded 97% for all diets, those containing a SBM : SPC ratio of 1 : 1 with 102 or 80 g kg^{-1} of lipid content reached the lowest ACLD coefficients among all diets ($P < 0.05$). On the other hand, diets with 102 g kg^{-1} crude lipid and a SBM : SPC ratio of 1 : 2.3 or 2.5 : 1 resulted in nearly 99% ACLD, significantly higher than the control diet ($P < 0.05$, Tukey's HSD).

Apparent digestibility of gross energy (AGED) in plant-based diets ranged from 72.7 ± 5.00% to 77.6 ± 0.56% compared with 79.3 ± 1.75% achieved with the control diet. There was no significant effect of SBM : SPC ratio or the dietary lipid content over the apparent gross energy digestibility coefficients ($P > 0.05$, two-way ANOVA). However, a significant correlation was observed between ACPD and AGED ($P < 0.05$, $R^2 = 0.80$).

Table 3 Performance of *Litopenaeus vannamei* fed on plant-based diets for 73 days under a clear-water rearing system starting with 3.97 ± 0.52 g (n = 500)

Performance	SBM : SPC ratio	Dietary lipid level (g kg^{-1} of the diet, dry matter basis)		
		121	102	80
Final survival (%)	1 : 2.3	85.4 ± 8.3	88.3 ± 4.0	87.3 ± 4.4
	1 : 1	84.4 ± 8.0*	86.3 ± 3.7	86.3 ± 3.7
	2.5 : 1	89.3 ± 3.7	84.9 ± 5.3*	84.4 ± 5.6*
	Control	–	–	94.1 ± 4.1*
Growth (g week^{-1})	1 : 2.3	0.75 ± 0.09	0.74 ± 0.04	0.70 ± 0.14
	1 : 1	0.72 ± 0.13	0.71 ± 0.14	0.71 ± 0.13
	2.5 : 1	0.71 ± 0.02	0.72 ± 0.12	0.71 ± 0.07
	Control	–	–	0.75 ± 0.13
Gained yield (g m^{-2})	1 : 2.3	530 ± 92	555 ± 58	512 ± 102
	1 : 1	500 ± 90	514 ± 119	512 ± 107
	2.5 : 1	538 ± 40	507 ± 120	493 ± 74
	Control	–	–	606 ± 86
Feed intake (g shrimp^{-1})	1 : 2.3	17.8 ± 1.5	16.5 ± 1.3	16.2 ± 1.3
	1 : 1	16.6 ± 1.7	16.3 ± 2.7	17.4 ± 0.6
	2.5 : 1	16.5 ± 0.6	16.8 ± 0.2	17.5 ± 0.5
	Control	–	–	16.4 ± 0.6
FCR	1 : 2.3	2.48 ± 0.64	2.14 ± 0.28	2.33 ± 0.53
	1 : 1	2.44 ± 0.53	2.30 ± 0.36	2.52 ± 0.59
	2.5 : 1	2.20 ± 0.19	2.50 ± 0.77	2.57 ± 0.42
	Control	–	–	1.96 ± 0.33

Two-way ANOVA	SBM : SPC ratio	Lipid content	SBM : SPC ratio × lipid content
Final survival	0.806	0.969	0.484
Growth	0.866	0.851	0.991
Gained yield	0.751	0.822	0.936
Feed intake	0.950	0.576	0.238
FCR	0.811	0.695	0.687

SBM, soybean meal; SPC, soy protein concentrate; FCR, food conversion ratio.
* Statistically significant difference between the experimental diet and the control (P < 0.05) according two-tailed t-tests.

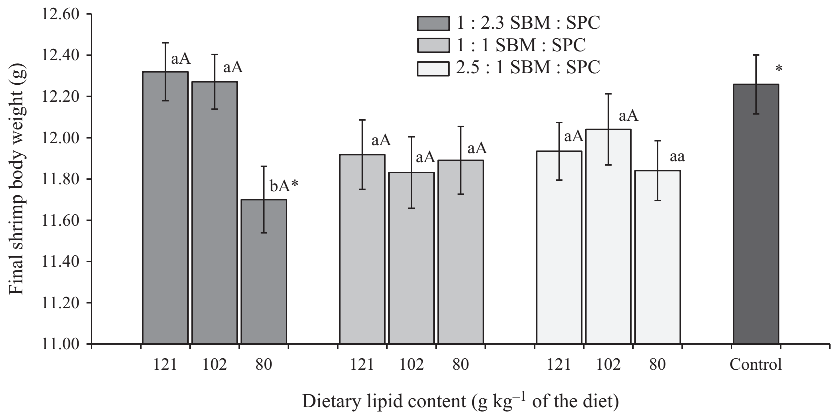

Figure 1 Final body weight of juveniles of *Litopenaeus vannamei* fed on plant protein-based diets for 73 days under a clear-water rearing system. Common letters denote non-statistically significant differences between experimental diets at the α = 0.05 level by Tukey's HSD. Capital letters refer to comparisons between different SBM:SPC ratios within the same dietary lipid content, while lowercase the vice versa. Asterisk indicates statistically significant difference between the experimental diet and the control (P < 0.05). SBM, soybean meal; SPC, soy protein concentrate.

Table 4 Apparent digestibility of protein, lipid and energy in plant-based diets for juveniles of *Litopenaeus vannamei*

		Dietary lipid level (% of the diet, dry matter basis)		
Digestibility (%)	SBM : SPC ratio	121	102	80
Protein	1 : 2.3	71.3 ± 1.53 aA*	73.2 ± 2.07 aA	75.7 ± 1.50 aA
	1 : 1	77.3 ± 2.54 aA	77.2 ± 2.04 aAB	78.5 ± 2.90 aA
	2.5 : 1	72.0 ± 6.62 aA	78.0 ± 0.48 aB	77.3 ± 5.86 aA
	Control	–	–	82.1 ± 2.42*
Lipid	1 : 2.3	97.0 ± 0.88 aA	98.8 ± 0.23 bA*	98.7 ± 0.31 abA
	1 : 1	98.6 ± 0.18 aA	97.7 ± 0.08 bcB	97.8 ± < 0.001 cB
	2.5 : 1	98.3 ± 0.41 aA	98.9 ± 0.16 aA*	98.7 ± 0.12 aA
	Control	–	–	97.5 ± 0.50*
Energy	1 : 2.3	72.7 ± 5.00 aA	77.2 ± 2.96 aA	76.4 ± 1.06 aA
	1 : 1	77.6 ± 0.56 aA	77.1 ± 1.28 aA	77.3 ± 4.17 aA
	2.5 : 1	73.7 ± 7.01 aA	77.5 ± 0.47 aA	75.9 ± 6.23 aA
	Control	–	–	79.3 ± 1.75

Two-way ANOVA	SBM : SPC ratio	Lipid content	SBM : SPC ratio × lipid content
Protein	0.030	0.118	0.359
Lipid	0.016	0.020	<0.0001
Energy	0.595	0.398	0.743

SBM, soybean meal; SPC, soy protein concentrate.
Common letters denote non-statistically significant differences between diets at the $\alpha = 0.05$ level by Tukey's HSD. Capital letters refer to comparisons between SBM : SPC ratios within the same dietary lipid content, while lowercase the vice versa.
* Statistically significant difference between the experimental diet and the control ($P < 0.05$) according two-tailed *t*-tests.

There was a significant improvement in shrimp growth, gained yield, feed intake and FCR when krill meal was used in plant-based diets at inclusion levels above 10 g kg^{-1} (Table 5; $P < 0.05$, one-way ANOVA). Shrimp final survival exceeded 93%, but no effect could be related to krill meal inclusion ($P > 0.05$, one-way ANOVA). However, shrimp growth was significantly improved from 0.90 ± 0.06 g to 1.00 ± 0.05 g week^{-1} when dietary inclusion of krill meal increased from 10 to 20 g kg^{-1} ($P < 0.05$). Over this range, gained yield raised 17.6%, from 608 ± 39 to 715 ± 31 g m^{-2}. Shrimp feed intake was also significantly enhanced at a dietary inclusion of 10 g kg^{-1} krill meal, while at 20 g kg^{-1} it was able to reduce FCR ($P < 0.05$, Tukey's HSD).

Shrimp final body weight was improved only at a dietary inclusion of 20 g kg^{-1} krill meal (Fig. 2; $P < 0.05$, one-way ANOVA). At this level, shrimp final body weight increased from 12.13 ± 1.97 g (10 g kg^{-1} krill meal) to 13.12 ± 1.88 g kg^{-1}. No enhancement in shrimp final body weight could be observed beyond this inclusion level.

Discussion

The present study has demonstrated that juveniles of *L. vannamei* can be fed and raised under intensive clear-water conditions on diets comprised almost entirely of plant ingredients without deleterious effects to their survival and to some extent upon growth. Shrimp diets containing high inclusion levels of plant ingredients are usually not perceived as good performers. This is probably due to the presence of antinutritional factors in plant feedstuffs, their deficiency in key essential amino acids and fatty acids, and potential lower protein, amino acid, and phosphorous bioavailability (Gatlin *et al.* 2007). However, other works carried out with *L. vannamei* have also shown that as long as diets are appropriately supplemented with specific additives such as *n*-3 LC-PUFA lipid sources, synthetic amino acids and feeding stimulants, soy proteins can fully replace fish meal without adverse effects to shrimp survival and growth performance (Amaya *et al.* 2007a,b; Fox *et al.* 2011; Browdy *et al.* 2012; Sá *et al.* 2013).

Juveniles of the white shrimp, *L. vannamei*, can thrive feeding on diets made up almost entirely of soybean and other plant protein sources. Amaya *et al.* (2007a) were the first to report the possibility of raising *L. vannamei* on plant-based diets. Authors formulated a diet containing a sum of 863 g kg^{-1} of plant ingredients (combination of SBM, sorghum, corn gluten meal and corn-fermented solubles) and evaluated against four other diets with 160 g kg^{-1} poultry by-product meal that progressively replaced Menhaden fish meal by SBM. After 81 days,

Table 5 Performance of *Litopenaeus vannamei* fed on plant-based diets containing minimum levels of krill meal. Shrimp were raised for 72 days under a clear-water rearing system

Diets[1]	Survival[2] (%)	Growth[2] (g week^{-1})	Gained yield[2] (g m^{-2})	Feed Intk.[2] (g shrimp^{-1})	FCR[2]
0	96.9 ± 2.4	0.86 ± 0.01 a	595 ± 21 a	15.5 ± 0.4 a	1.83 ± 0.09 ab
5	93.1 ± 5.2	0.86 ± 0.03 a	566 ± 46 a	15.6 ± 1.3 a	1.93 ± 0.07 a
10	95.0 ± 5.4	0.90 ± 0.06 a	608 ± 39 a	16.1 ± 1.1 ab	1.86 ± 0.09 ab
20	99.4 ± 1.3	1.00 ± 0.05 b	715 ± 31 b	17.2 ± 1.3 ab	1.69 ± 0.09 b
30	97.5 ± 2.0	1.05 ± 0.05 b	732 ± 36 b	18.2 ± 0.7 b	1.75 ± 0.05 b
P^2	0.200	<0.0001	<0.0001	0.004	0.008

FCR, food conversion ratio.
Common letters denote non-statistically significant differences between diets at the α = 0.05 level by Tukey's HSD. Shrimp were stocked with 2.84 ± 0.52 g (*n* = 800).
[1] Values indicate the dietary inclusion of krill meal (g kg^{-1} of the diet, as is basis).
[2] One-way ANOVA.

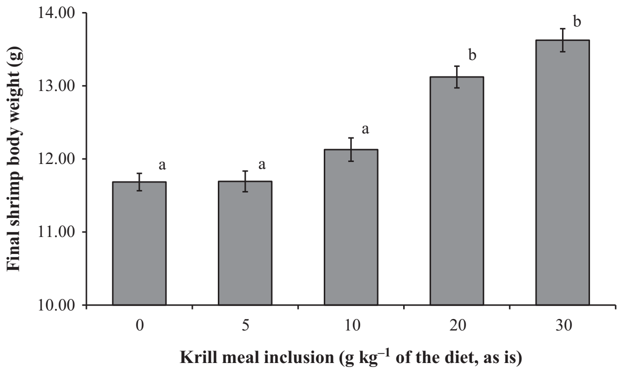

Figure 2 Final body weight of juveniles of *Litopenaeus vannamei* fed on plant protein-based diets with minimum levels of krill meal for 72 days under a clear-water rearing system. Common letters denote non-statistically significant differences between experimental diets at the α = 0.05 level by Tukey's HSD.

L. vannamei final survival, growth performance and FCR under green-water tank conditions were not significantly different among dietary treatments.

Sookying *et al.* (2011) reared juveniles of the white shrimp in green-water tanks and in lined ponds under stocking densities of up to 65 shrimp m^{-2}. Shrimp were fed diets that contained from 500 to more than 800 g kg^{-1} of their composition based on terrestrial plant ingredients, mainly SBM. After 16 weeks of pond rearing, shrimp achieved from 58.0% to 65.1% final survival, from 20.70 to 25.25 g final body weight, and from 1.17 to 1.54 FCR. After a 10-week rearing period in tanks, Sookying *et al.* (2011) reported that shrimp final survival, body weight and FCR reached between 93.4 ± 5.6% and 100.0 ± 0.0%, 13.42 and 16.13 g, and 1.15 and 1.54, respectively. In another study, Sookying & Davis (2011) raised *L. vannamei* for 18 weeks in lined ponds of 0.1 ha under 35 shrimp m^{-2}. Authors found no difference in shrimp performance between those fed a high SBM diet, up to 580 g kg^{-1}, versus another one containing 100 g kg^{-1} Menhaden fish meal. Roy *et al.* (2009) used the same diets to raise *L. vannamei* under low salinity conditions (4 g L^{-1}) in laboratory and farm conditions and reported similar results. Morris *et al.* (2011) fed juveniles of *L. vannamei* for 12 weeks in green-water tanks with free fish meal diets containing 605 g kg^{-1} SBM and 80 g kg^{-1} corn gluten meal. Authors evaluated the differences between cholesterol- and phytosterol-supplemented diets. They predicted that cholesterol requirement for maximum growth of *L. vannamei* fed on plant-based diets to be 15 g kg^{-1}.

While studies by Amaya *et al.* (2007a), Sookying *et al.* (2011), Sookying & Davis (2011), Roy *et al.* (2009), and Morris *et al.* (2011) were carried out under green water, in tanks and (or) under pond conditions, the present work was conducted in clear-water tanks with diets exceeding 900 g kg^{-1} of their composition of plant ingredients. While one may argue that previous work was carried out in the presence of naturally occurring food items which shrimp may nourish on, in the present study, animals had no or little access to natural food sources other than the experimental diets. Thus, it is clear that young juveniles of *L. vannamei* can rapidly adapt and sustain their growth with plant-based diets even in the lack of natural food items in water.

In the present work, both the SBM : SPC ratio and the dietary lipid content of diets had little effect on shrimp growth performance. Shrimp final body weight was only depressed when a dietary lipid content of 80 g kg^{-1} was used in combination with the highest levels of SPC to SBM. Up to 102 g kg^{-1} dietary lipid, there was a non-statistically significant trend towards improved final body weight and yield with as much as 345 g kg^{-1} of SPC. Contradictorily, at this dietary lipid content, ACPD decreased with higher levels of SPC. Crude protein from different soy sources may exhibit comparable apparent protein digestibility coefficients for juvenile *L. vannamei* as predicted by *in vitro* and verified by *in vivo* methods (Lemos *et al.* 2009, 2011). On the other hand, salmon by-product meal is among the highest digestible fish meal proteins. This may have led to superior ACPD in the control diet. Lack of significant difference in apparent gross energy digestibility (AGED) between diets with different soy protein ratios at dietary nitrogen-free extract above 40% is surprising as SPC contains a lower crude fibre content than SBM. Present ACPD and AGED data at levels between 67% and 82% may be attributed to reduced leaching of diet and faeces by use of automated feeders and regular sampling, respectively (Smith & Tabrett 2004; Carvalho 2011).

There is also potential to make use of other plant derived feedstuffs, such as pea meal (Cruz-Suárez *et al.* 2001; Davis *et al.* 2002; Bautista-Teruel *et al.* 2003; Sookying & Davis 2011) and canola meal (Cruz-Suárez *et al.* 2001, 2009; Bulbul *et al.* 2013) in plant-based diets alone or in combination with soy proteins. However, at the moment, these may not be as commercially available and price competitive as soy proteins. As in the case of corn gluten meal, dietary inclusions should be further restrained due to suboptimal protein and amino acid digestibility.

In the present work, in order to accommodate higher dietary levels of SBM, inclusion of corn gluten meal increased from an average of 64.7–80.0 g kg^{-1}. However, this dietary range did not seem to cause any loss to shrimp growth performance or feed digestibility. Similar dietary inclusion levels, between 60 and 80 g kg^{-1} of the diet, have been adopted in other works with *L. vannamei* without any reports of deleterious growth effects (González-Félix *et al.* 2010; Morris *et al.* 2011). However, other works have reported that the use of corn gluten leads to a deleterious growth performance and poor apparent protein digestibility in *L. vannamei*. Molina-Poveda *et al.* (2015) reported a reduced palatability in diets for juvenile *L. vannamei* when a dietary reduction in fish meal from 329.1 to 246.8 g kg^{-1} was accompanied by an increase in corn gluten meal, from 0 to 86.4 g kg^{-1}. Authors associated a poor shrimp growth, an increased FCR, and a lower protein and essential amino acid digestibility with progressive increases in the dietary inclusion of corn gluten meal, from 0 to 345.6 g kg^{-1}. Molina-Poveda *et al.* (2015) diets contained a minimum inclusion of corn gluten meal of 86.4 g kg^{-1}, nearly equivalent to the highest inclusions adopted in this study (i.e. 80.0 g kg^{-1}).

Molina-Poveda *et al.* (2015) findings were supported by Carvalho (2011) and Lemos *et al.* (2009). Carvalho (2011) worked on the apparent digestibility of a corn gluten meal for *L. vannamei* containing 660.9 g kg^{-1} crude protein and 31.7 g kg^{-1} methionine (Met). The author found an apparent protein and methionine digestibility for corn gluten meal in the range of 46.6% and 59.9%, and from 61.9% to 71.0%, respectively, the lowest among five other ingredients (anchovy meal, SPC, poultry by-product meal, meat and bone meal, hydrolysed feather meal). Similarly, Lemos *et al.* (2009) reported an ACPD for corn gluten meal of 59.1 ± 1.9%.

In this study, it was possible to establish that a minimum dietary inclusion of 20 g kg^{-1} of krill meal in a plant-based diet was able to significantly enhance the growth, yield, FCR and final body weight of juvenile *L. vannamei*. At 10 g kg^{-1}, krill meal only improved feed intake. Dietary levels beyond 20 g kg^{-1} brought no benefit to shrimp performance. For *Litopenaeus stylirostris*, Suresh *et al.* (2011) found that krill meal at 30 g kg^{-1} acted as an effective attractant, palatability enhancer as well as growth enhancer in diets having no fish meal, formulated with high levels of poultry by-product meal (200 g kg^{-1}) and SBM (400 g kg^{-1}).

Previous studies had also demonstrated that the benefit of krill meal in low fish meal diets for marine shrimp goes beyond improving feed attractability. Nunes *et al.* (2011) was able to fully replace the protein and lipid value of fish meal, fish oil, soybean lecithin and cholesterol at no cost to performance using krill meal in *L. vannamei* diets. Sá *et al.* (2013) fed juveniles of *L. vannamei* with diets free from fish meal containing a low inclusion of squid and krill meal (from 5 to 20 g kg^{-1} in total). Authors worked with diets that contained a fixed amount of SBM to SPC (330–50 g kg^{-1}, respectively), and a decreasing dietary level of poultry by-product meal, starting at 150 g kg^{-1}. Results indicated a significant increment in final shrimp body weight when a combination of 20 g kg^{-1} krill meal and squid meal was used.

Conclusions

Results from present study have shown that juvenile *L. vannamei* is able to survive and grow under clear-water feeding on diets made up almost entirely of plant ingredients (over 920 g kg^{-1}). No beneficial effect to shrimp performance and feed ACPD was identified by feeding animals on a diet formulated with a combination of animal (fish meal and meat and bone meal) and plant proteins versus diets with plant proteins alone. Shrimp performance did not respond significantly to soy protein ratio in plant-based diets. However, dietary lipid levels of 80 and 121 g kg^{-1} combined with a high SPC to SBM ratio should not be adopted as this resulted in a lower final body weight and poorer ACPD, respectively. Results have suggested that higher dietary levels of SPC to SBM accompanied by a crude lipid content of 102 g kg^{-1} may be more desirable to enhance the growth of juveniles of *L. vannamei*. This study has demonstrated that krill meal in plant-based diets enhanced feed intake at only 10 g kg^{-1} of the diet. At 20 g kg^{-1}, krill meal accelerated shrimp growth, while increasing yield and reducing FCR.

Acknowledgements

The last author acknowledges the support from a research productivity fellowship (CNPq/MCT, PQ# 305513/2012-5). Research support from Ricardo H. Ota, FAPESP (2007/07051-9) and CNPq (304831/2009-3) for the digestibility study in USP are appreciated (D. Lemos).

References

Alvarez, J.S., Hernández-Llamas, A., Galindo, J., Fraga, I., García, T. & Villarreal, H. (2007) Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp *Litopenaeus schmitti* (Pérez-Farfante & Kensley 1997). *Aquacult. Res.*, **38**, 689–695.

Amaya, E., Davis, D.A. & Rouse, D.B. (2007a) Alternative diets for the Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, **262**, 419–425.

Amaya, E., Davis, D.A. & Rouse, D.B. (2007b) Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. *Aquaculture*, **262**, 393–401.

AOAC (2002) Official Methods of Analysis of AOAC International, 17th edn. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, W. Jr & Poersch, L.H.S. (2012) Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, **342–343**, 112–116.

Bautista-Teruel, M.N., Eusebio, P.S. & Welsh, T.P. (2003) Utilization of feed pea, *Pisum sativum*, meal as a protein source in practical diets for juvenile tiger shrimp, *Penaeus monodon*. *Aquaculture*, **225**, 121–131.

Browdy, C.L., Bharadwaj, A.S., Venero, J.A. & Nunes, A.J.P. (2012) Supplementation with 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) in low fish meal diets for the white shrimp, *Litopenaeus vannamei*. *Aquacult. Nutr.*, **18**, 432–440.

Bulbul, M., Koshio, S., Ishikawa, M., Yokoyama, S. & Kader, M.A. (2013) Performance of kuruma shrimp, *Marsupenaeus japonicus* fed diets replacing fishmeal with a combination of plant protein meals. *Aquaculture*, **372–375**, 45–51.

Bulbul, M., Kader, M.A., Koshio, S., Ishikawa, M. & Yokoyama, S. (2014) Effect of replacing fishmeal with canola meal on growth and nutrient utilization in kuruma shrimp *Marsupenaeus japonicus* (Bate). *Aquacult. Res.*, **45**, 848–858.

Bulbul, M., Kader, M.A., Ambak, M.A., Hossain, M.S., Ishikawa, M. & Koshio, S. (2015) Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, *Marsupenaeus japonicus*. *Aquaculture*, **438**, 96–104.

Carvalho, R.A.P.L.F. (2011) Desenvolvimento de um sistema de recirculação para estudos sobre digestibilidade em condições de alto desempenho para camarões marinhos: avaliação de ingredientes proteicos alternativos à farinha de peixe em diferentes níveis de inclusão em dietas para juvenis de *Litopenaeus vannamei*. Doctoral Thesis. Instituto Oceanográfico da Universidade de São Paulo, São Paulo, Brazil.

Carvalho, R.A.P.L.F., Lemos, D.E.L. & Tacon, A.G.J. (2013) Performance of single-drain and dual-drain tanks in terms of water velocity profile and solids flushing for *in vivo* digestibility studies in juvenile shrimp. *Aquacult. Eng.*, **57**, 9–17.

Cruz-Suárez, L.E., Ricque-Marie, D.M., Tapia-Salazar, M., McCallum, I.M. & Hickling, D. (2001) Assessment of differently processed feed pea (*Pisum sativum*) meals and canola meal (*Brassica* sp.) in diets for blue shrimp (*Litopenaeus stylirostris*). *Aquaculture*, **196**, 87–104.

Cruz-Suárez, L.E., Tapia-Salazar, M., Villarreal-Cavazos, D., Beltran-Rocha, J., Nieto-López, M.G., Lemme, A. & Ricque-Marie, D. (2009) Apparent dry matter, energy, protein and amino acid digestibility of four soybean ingredients in white shrimp *Litopenaeus vannamei* juveniles. *Aquaculture*, **292**, 87–94.

Davis, D.A., Arnold, C.R. & McCallum, I. (2002) Nutritional value of feed peas (*Pisum sativum*) in practical diet formulations for *Litopenaeus vannamei*. *Aquacult. Nutr.*, **8**, 87–94.

Forster, I.P. & Dominy, W.G. (2006) Efficacy of three methionine sources in diets for Pacific white shrimp, *Litopenaeus vannamei*. *J. World Aquacult. Soc.*, **37**, 474–480.

Fox, J.M., Humes, M., Davis, D.A. & Lawrence, A.L. (2011) Evaluation of methionine supplements and their use in grain-based feeds for *Litopenaeus vannamei*. *J. World Aquacult. Soc.*, **42**, 676–686.

Francis, G., Makkar, H.P.S. & Becker, K. (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**, 197–227.

Gatlin, D.M. III, Barrows, F.T., Brown, P. *et al.* (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.*, **38**, 551–579.

Gong, H., Lawrence, A.L., Jiang, D.H., Castille, F.L. & Gatlin, D.M. III (2000) Lipid nutrition of juvenile *Litopenaeus vannamei* I. Dietary cholesterol and de-oiled soy lecithin requirements and their interaction. *Aquaculture*, **190**, 305–324.

González-Félix, M.L., Silva, F.S.D., Davis, D.A., Samocha, T.M., Morris, T.C., Wilkenfeld, J.S. & Perez-Velazquez, M. (2010) Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*, **309**, 152–158.

Gu, M., Zhang, W.B., Bai, N., Mai, K.S. & Xu, W. (2013) Effects of dietary crystalline methionine or oligo-methionine on growth performance and feed utilization of white shrimp (*Litopenaeus vannamei*) fed plant protein-enriched diets. *Aquacult. Nutr.*, **19**, 39–46.

Lemos, D., Lawrence, A.L. & Siccardi, A.J. III (2009) Prediction of apparent protein digestibility of ingredients and diets by *in vitro* pH-stat degree of protein hydrolysis with species-specific enzymes for juvenile Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, **295**, 89–98.

Lemos, D., Yasumaru, F., Carvalho, R. & Tacon, A.G.J. (2011) Application of Species-Specific *In Vitro* Digestion to Access Protein Digestibility in Shrimp Feeds: Current Status and Prospects. World Aquaculture, The World Aquaculture Society, Natal, Brazil.

Molina-Poveda, C., Lucas, M. & Jover, M. (2013) Evaluation of the potential of Andean lupin meal (*Lupinus mutabilis* Sweet) as an alternative to fish meal in juvenile *Litopenaeus vannamei* diets. *Aquaculture*, **410**, 148–156.

Molina-Poveda, C., Lucas, M. & Jover, M. (2015) Utilization of corn gluten meal as a protein source in the diet of white shrimp *Litopenaeus vannamei*. *Aquacult. Nutr*, **21**, 824–834.

Morris, T.C., Samocha, T.M., Davis, D.A. & Fox, J.M. (2011) Cholesterol supplements for *Litopenaeus vannamei* reared on plant based diets in the presence of natural productivity. *Aquaculture*, **314**, 140–144.

National Research Council (NRC) (2011) Nutrient Requirements of Fish and Shrimp, 376 pp. Animal Nutrition Series. National Research Council of the National Academies, The National Academies Press, Washington, DC, USA.

Nunes, A.J.P., Sá, M.V.C., Carvalho, E.A. & Sabry-Neto, H. (2006) Growth performance of the white shrimp *Litopenaeus vannamei* reared under time- and rate-restriction feeding regimes in a controlled culture system. *Aquaculture*, **253**, 646–652.

Nunes, A.J.P., Sá, M.V.C. & Sabry-Neto, H. (2011) Growth performance of the white shrimp, *Litopenaeus vannamei*, fed on practical diets with increasing levels of the Antarctic krill meal, *Euphausia superba*, reared in clear- versus green-water culture tanks. *Aquacult. Nutr.*, **17**, e511–e520.

Nunes, A.J.P., Sá, M.V.C., Browdy, C.L. & Vázquez-Añón, M. (2014) Practical supplementation of shrimp and fish feeds with crystalline amino acids. *Aquaculture*, **431**, 20–27.

Oujifard, A., Seyfabadi, J., Abedian-Kenari, A. & Rezaei, M. (2015) Growth response and tail-muscle fatty acid quality of Pacific white shrimp, *Litopenaeus vannamei* (Boone) fed with diets containing different levels of rice protein concentrate. *Iran. J. Fish. Sci.*, **14**, 188–200.

Paripatananont, T., Boonyaratpalin, M., Pengseng, P. & Chotipuntu, P. (2001) Substitution of soy protein concentrate for fishmeal in diets of tiger shrimp *Penaeus monodon*. *Aquacult. Res.*, **32**(Suppl. 1), 369–374.

Roy, L.A., Bordinhon, A., Sookying, D., Davis, D.A., Brown, T.W. & Whitis, G.N. (2009) Demonstration of alternative feeds for the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters of west Alabama. *Aquacult. Res.*, **40**, 496–503.

Sá, M.V.C., Sabry-Neto, H., Cordeiro-Júnior, E. & Nunes, A.J.P. (2013) Dietary concentration of marine oil affects replacement of fish meal by soy protein concentrate in practical diets for the white shrimp, *Litopenaeus vannamei*. *Aquacult. Nutr.*, **19**, 199–210.

Silva-Neto, J.F., Nunes, A.J.P., Sabry-Neto, H. & Sá, M.V.C. (2012) Spirulina meal has acted as a strong feeding attractant for *Litopenaeus vannamei* at a very low dietary inclusion level. *Aquacult. Res.*, **43**, 430–437.

Smith, D.M. & Tabrett, S.J. (2004) Accurate measurement of *in vivo* digestibility of shrimp feeds. *Aquaculture*, **232**, 564–580.

Sookying, D. & Davis, A. (2009) Growth of Pacific white shrimp *Litopenaeus vannamei* fed diets containing different levels of soy protein concentrate reared under clear water system. *J. Shellfish Res.*, **28**, 730.

Sookying, D. & Davis, A. (2011) Pond production of Pacific white shrimp (*Litopenaeus vannamei*) fed high levels of soybean meal in various combinations. *Aquaculture*, **319**, 141–149.

Sookying, D., Silva, F.S.D., Davis, D.A. & Hanson, T.R. (2011) Effects of stocking density on the performance of Pacific white shrimp *Litopenaeus vannamei* cultured under pond and outdoor tank conditions using a high soybean meal diet. *Aquaculture*, **319**, 232–239.

Sookying, D., Davis, D.A. & Silva, F.S.D. (2013) A review of the development and application of soybean-based diets for Pacific white shrimp *Litopenaeus vannamei*. *Aquacult. Nutr.*, **19**, 441–448.

Suresh, A.V., Kumaraguru Vasagam, K.P. & Nates, S. (2011) Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal. *Aquaculture*, **319**, 132–140.