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Dietary concentration of marine oil affects replacement of fish meal by soy protein concentrate in practical diets for the white shrimp, *Litopenaeus vannamei*

M.V.C. SÁ[1,2], H. SABRY-NETO[1], E. CORDEIRO-JÚNIOR[2] & A.J.P. NUNES[1]

[1] *LABOMAR – Instituto de Ciências do Mar, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil;* [2] *Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil*

Abstract

This work aimed to determine whether a minimum provision of marine oil in practical diets for *Litopenaeus vannamei* is required when replacing fish meal (FM) by soy protein concentrate (SPC). The study consisted of three growth experiments conducted in 500-L tanks with 70 shrimp m^{-2}. In experiment #1, FM was progressively replaced by SPC as fish oil (FO) levels increased with a consistent input of whole squid meal (WSM). In experiment #2, FM was replaced by SPC under two levels of FO (10 or 20 g kg^{-1}) without the presence of a feeding effector. In experiment #3, three dietary levels of krill meal (KRL) and WSM (5, 10 and 20 g kg^{-1}) were included in a basal diet containing SPC and low levels of FM. Results showed that under a clear-water condition, the dietary levels of FO in practical diets for *L. vannamei* have a significant impact on the amount of FM that can be replaced by SPC. As much as 31% replacement of FM/SPC was possible with 20 g kg^{-1} FO. Whenever dietary fat was adjusted by using FO as a lipid source, complete replacement of FM by SPC was achieved with no negative effect on shrimp growth.

KEY WORDS: fish meal, marine oil, shrimp, SPC

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Correspondence: Alberto 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: albertojpn@uol.com.br

Introduction

Soybean is the most significant oilseed produced globally with annual harvests exceeding 220 million metric tons (Masuda & Goldsmith 2009). About one-fourth of this output is converted into meals and cakes for use within the animal feed industry. In aquatic feeds, soybean meal (SBM), soy protein concentrate (SPC) and soy protein isolate are all regarded as viable protein alternatives to replace fish meal (FM) in industrially manufactured feeds for farm-raised fish and crustaceans (Gatlin *et al.* 2007).

In comparison with FM, soy-derived proteins are methionine deficient, contain low levels of available phosphorus and have antinutritional factors (Gatlin *et al.* 2007) and poor palatability property (Nunes *et al.* 2006a). SBM is by far the least expensive and most common soy protein used in shrimp diets. Recently, dietary levels as high as 580 g kg^{-1} has proven successful in the semi-intensive culture of the white shrimp, *Litopenaeus vannamei* (Sookying & Davis 2011; Sookying *et al.* 2011).

Soy protein concentrate is a more refined soy product than SBM because soluble carbohydrates are removed during processing and antinutritional factors reduced. Dietary use of SPC in aquafeeds is less common than SBM, but there has been a recent growing interest (Cruz-Suárez *et al.* 2009; Salze *et al.* 2010; Carter & Sajjadi 2011). While amino acid and phosphorous deficiency of SPC are well known and properly addressed in feed formulation, the minimum levels of marine oil in SPC-based diets required to successfully replace FM has received little attention. Penaeids have a limited ability for synthesizing long-chain polyunsaturated fatty acids (LC-PUFA; Kanazawa *et al.* 1979; Glencross & Smith 2001; Glencross 2009), and thus, a minimum provision of essential fatty acids in their diets appears to be required. This work aimed at determining whether a minimum dietary inclusion of marine oil is required to achieve successful replacement FM by SPC in practical diets for the white shrimp, *L. vannamei*.

Material and methods

Experimental design, shrimp and culture system

The study was divided into three growth experiments with the Pacific white shrimp, *L. vannamei*, which were all carried out at the marine aquaculture laboratory facilities of LABOMAR (Eusebio, Ceará, Brazil). In all three studies, the effects of FM replacement by SPC on shrimp growth performance were evaluated under different dietary inclusion levels of fish oil (FO) and/or feeding effectors. In experiment #1, FM was progressively replaced SPC as FO levels were increased under a consistent input of whole squid meal (WSM at 20 g kg^{-1}). In experiment #2, FM was replaced by SPC under two consistent levels of FO (10 or 20 g kg^{-1}) without the presence of a feeding effector. In experiment #3, three dietary levels of krill meal (KRL) and WSM (5, 10 and 20 g kg^{-1}) were included in a basal diet containing 77.5 g kg^{-1} of SPC and low levels of FM (50 g kg^{-1}).

Non-SPF (Specific Pathogen-Free) shrimp were brought to the laboratory as PL12 (12-day-old postlarvae) from a commercial hatchery (Speed Line BR strain; Aquatec Industrial Pecuária Ltda., Canguaretama, Rio Grande do Norte, Brazil). A total of 20 000 animals were transported by land at a density of 666 post-larvae L^{-1}, sheltered from sunlight in four double plastic bags containing *Artemia* spp. and dissolved oxygen. At arrival to the laboratory, shrimp population was split evenly and stocked at 2.4 PL L^{-1} in six round nursery tanks of 3000 L. Over the nursery phase, shrimp were fed eight times per day (at 07:00, 09:00, 11:00, 13:00, 15:00, 17:00, 23:00 and 03:00 h) on a laboratory-made crumbled diet (400 g kg^{-1} crude protein, 95 g kg^{-1} fat, 13 g kg^{-1} fibre, 53 g kg^{-1} ash and 17.7 kJ g^{-1} gross energy).

After reaching 1.0 g in body weight, juvenile shrimp were transferred to outdoor tanks of 1000 L in volume (1.02 m^2 of bottom surface area) and stocked under 250 shrimp m^{-2}. The nursery phase lasted from 30 to 45 days when shrimp attained between 1.59 ± 0.46 g (n = 100; experiments #2 and #3) and 3.36 ± 0.80 g (n = 30; experiment #1) in wet body weight. At this time, shrimp were size-graded, counted, individually weighed and stocked at 40 shrimp $tank^{-1}$ (70 shrimp m^{-2}) in 500 L clear-water tanks (0.57 m^{-2} of bottom surface area). Water quality management, shrimp feeding and sampling followed a similar protocol as described by Nunes *et al.* (2011).

In growth experiments #1, 2 and 3, a total of 25, 48 and 20 indoor tanks were used, respectively, allowing from five to six replicate tanks per diet. Shrimp were allowed to acclimate in the rearing system for 14 days feeding on a commercial shrimp diet (Camaronina 35 HP; Evialis do Brasil Nutrição Animal Ltda., São Lourenço da Mata, Pernambuco, Brazil).

Diets

For experiment #1, a basal diet was designed to contain 180 g kg^{-1} of FM (ratio of 5 : 1 of anchovy FM to bycatch FM). From the basal diet, four other isonitrogenous diets were formulated to replace FM by SPC at 25%, 50%, 75% and 100%. As FM was withdrawn, dietary levels of FO were raised from 14.3 g kg^{-1} in the basal diet to a maximum of 25.0 g kg^{-1} in the diet deprived of FM. WSM was included at 20 g kg^{-1} in all diets subjected to FM replacement. Crystalline DL-methionine was supplemented at 0.5 and 1.0 g kg^{-1} only in diets with 75% and 100% replacement of FM. Substitution of FM for SPC occurred also at the cost of broken rice and poultry by-product meal (PBM) (Table 1).

Crude protein, total lipid and gross energy levels in diets used in experiment #1 varied 1.8%, 2.9% and 1.5%, respectively, from 381 to 393 g kg^{-1}, 75 to 80 g kg^{-1} and 19.5 to 20.2 MJ kg^{-1}, respectively. Amino acid profile was consistent among experimental diets (Table 2). Average dietary amino acid variation was 4.1%, except for cystine, methionine and tryptophan, which varied from 10.3% to 12.0% among experimental diets (Table 2).

For experiment #2, two sets of four isonitrogenous diets (from 384.1 to 393.9 g of crude protein kg^{-1}) containing 10 or 20 g kg^{-1} of FO were formulated to replace FM by SPC. In this case, Tacon & Metian's (2008) forecasts for FM use in industrially compounded shrimp feeds in years 2010, 2015 and 2020 were applied to define FM dietary levels. Starting at 120.0 g kg^{-1}, FM was reduced to 85.0 and 50.0 g kg^{-1} until complete withdrawal was achieved. As dietary levels of FM dropped, inclusion of SPC increased from 0 to 38.5, 77.5 to 133.4 g kg^{-1}, respectively (Table 3). Comparatively, soybean meal (330 g kg^{-1}), wheat flour (250 g kg^{-1}) and PBM (150 g kg^{-1}) levels were kept consistent throughout all diets. Diets containing 120 g kg^{-1} FM were used as control diets, regardless of FO inclusion.

Diets in experiment #2 were supplemented with crystalline DL-methionine and L-lysine to match similar amino acid concentrations achieved by the control diets. Amino acid variation reached <5% among experimental diets,

Table 1 Ingredient and chemical composition of diets used in experiment #1

Ingredients	Diets/composition (g kg^{-1} of the diet, as is)				
% Substitution FM/SPC[1]	0%	25%	50%	75%	100%
FM, anchovy[2]	150.0	112.5	75.0	37.5	0.0
FM, by-catch[3]	30.0	22.5	15.0	7.5	0.0
Soy protein concentrate[4]	0.0	45.0	90.0	135.0	180.0
Broken rice[5]	120.0	124.4	117.8	111.0	104.2
Poultry by-product meal[6]	100.7	80.1	84.1	87.8	91.4
Fish oil[7]	14.3	19.0	21.6	24.2	25.0
Whole squid meal[8]	0.0	20.0	20.0	20.0	20.0
DL-methionine[9]	0.0	0.0	0.0	0.5	1.0
Soybean oil[10]	0.0	0.0	0.0	0.0	1.8
Magnesium sulphate	0.6	0.6	0.7	0.7	0.7
Potassium chloride	8.6	0.0	0.0	0.0	0.0
Others[11]	575.9	575.9	575.9	575.9	575.9
Proximate composition (g kg^{-1} of the diet, dry matter basis)					
Crude protein[12]	381	385	400	389	393
Crude fat[12]	75	77	81	77	79
Crude fibre[12]	14	14	17	39	44
Ash[12]	102	83	80	74	70
Nitrogen-free extract[13]	427	442	422	422	415
Gross energy[12] (MJ kg^{-1})	20	20	20	20	20

[1] Per cent substitution of fish meal (FM) for soy protein concentrate (SPC).
[2] COPEINCA Corporación Pesquera INCA S.A. (Lima, Peru). 677 g kg^{-1} crude protein (CP); 76 g kg^{-1} fat; 144 g kg^{-1} ash; 2 g kg^{-1} crude fibre (CF); 90 g kg^{-1} moisture.
[3] INPEL Indústria de Resíduos de Pescado Ltda. (Canoas, Santa Catarina, Brazil). 590 g kg^{-1} CP; 62 g kg^{-1} fat; 194 g kg^{-1} ash; 2 g kg^{-1} CF; 68 g kg^{-1} moisture.
[4] Sementes Selecta S.A. (Goiânia, Goiás, Brazil). 63 g kg^{-1} CP; 1 g kg^{-1} fat; 4 g kg^{-1} CF; 4 g kg^{-1} ash; 8 g kg^{-1} moisture.
[5] Usina Catende (Catende, Pernambuco, Brazil). 121 g kg^{-1} CP; 15 g kg^{-1} fat; 18 g kg^{-1} CF; 3 g kg^{-1} ash; 77 g kg^{-1} moisture.
[6] NORDAL Nordeste Indl. de Derivados Animais Ltda. (Maracanaú, Ceará, Brazil). 613 g kg^{-1} CP; 138 g kg^{-1} fat; 119 g kg^{-1} ash; 5 g kg^{-1} CF; 42 g kg^{-1} moisture.
[7] COPEINCA Corporación Pesquera INCA S.A. (Lima, Peru). 980 g kg^{-1} fat; 39 MJ kg^{-1} gross energy (GE).
[8] Hinrichsen Trading S.A. (Santiago, Chile). 689 g kg^{-1} CP; 54 g kg^{-1} fat; 116 g kg^{-1} ash; 5 g kg^{-1} CF; 109 g kg^{-1} moisture.
[9] DL-methionine feed grade 99%. Evonik Degussa Brasil Ltda. (São Paulo, São Paulo, Brazil).
[10] Perdigão. Bunge Alimentos S.A. (Luis Eduardo Magalhães, Bahia, Brazil). 996 g kg^{-1} fat; 39 MJ kg^{-1} GE.
[11] Others included: 300.0 g kg^{-1} of soybean meal[14], 200.0 g kg^{-1} of wheat flour[15], 30.0 g kg^{-1} of meat and bone meal[16], 15.0 g kg^{-1} of soybean lecithin[16], 10.0 g kg^{-1} of corn gluten meal[18], 10.0 g kg^{-1} of vitamin–mineral premix[19], 10.0 g kg^{-1} of common salt and 0.9 g kg^{-1} of ascorbic acid polyphosphate[20].
[12] Analysed values.
[13] Calculated by difference (1000 − CP − fat − CF − ash).
[14] Farelo de Soja 46. Bunge Alimentos S.A. (Luis Eduardo Magalhães, Bahia, Brazil). 466 g kg^{-1} CP; 13 g kg^{-1} fat; 63 g kg^{-1} ash; 43 g kg^{-1} CF; 90 g kg^{-1} moisture.
[15] Dona Benta Tipo 1, J. Macedo S.A. (Fortaleza, Ceará, Brazil). 109 g kg^{-1} CP; 5 g kg^{-1} fat; 5 g kg^{-1} ash; 3 g kg^{-1} CF; 143 g kg^{-1} moisture.
[16] NORDAL Nordeste Indl. de Derivados Animais Ltda. (Maracanaú, Ceará, Brazil). 463 g kg^{-1} CP; 165 g kg^{-1} fat; 311 g kg^{-1} ash; 6 g kg^{-1} CF; 72 g kg^{-1} moisture.
[17] Cargill Nutrição Animal Ltda. (Paulínia, São Paulo, Brazil). 928 g kg^{-1} fat; 61 g kg^{-1} ash; 34 MJ kg^{-1} GE.
[18] Protenose®, Corn Products Brasil (São Paulo, São Paulo, Brazil). 625 g kg^{-1} CP; 95 g kg^{-1} fat; 47 g kg^{-1} ash; 2 g kg^{-1} CF.
[19] Rovimix Camarao Intensivo, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, São Paulo, Brazil). Guarantee levels per kg of product: vitamin A, 1 250 000 IU; vitamin D3, 350 000 IU; vitamin E, 25 000 IU; vitamin K3, 500 mg; vitamin B1, 5000 mg; vitamin B2, 4000 mg; vitamin B6; 10 mg; nicotinic acid, 15 000 mg; pantothenic acid, 10 000 mg; biotin, 150 mg; folic acid, 1250 mg; vitamin C, 25 000 mg; cholin, 50 000 mg; inositol, 20 000 mg; iron 2000 mg; copper, 3500 mg; chelate copper, 15 000 mg; zinc, 10 500 mg; chelate zinc, 4500 mg; manganese, 4000 mg; selenium, 15 mg; chelate selenium, 15 mg; iodine, 150 mg; cobalt, 30 mg; chromium 80 mg; filler, 1000 g.
[20] Rovimix Stay-C® 35%, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, São Paulo, Brazil). L-ascorbic acid 2-monophosphate.

except alanine, cystine, serine, tryptophan and proline for which variations ranged from 5% to 10% (Table 4).

Diets in experiment #2 were made isoenergetic by increasing the dietary levels of soybean oil as inclusion of FM and FO was challenged. However, the total amount of LC-PUFA was allowed to freely drop. In general, dietary concentrations of eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic (22:6n-3; DHA) decreased in diets

Table 2 Amino acid profile of diets used in experiment #1 (g kg^{-1} of the diet, dry matter basis; analysed)

Amino acid % Substitution FM/SPC[1]	Diets (g kg^{-1}, dry matter basis)					
	0%	25%	50%	75%	100%	CV[2] (%)
Alanine	19.3	18.4	18.4	17.7	19.6	4.1
Arginine	23.5	23.5	24.7	24.1	25.6	3.7
Aspartic acid	33.2	33.5	35.2	35.0	36.9	4.3
Cystine	4.6	4.3	4.6	3.6	4.7	10.3
Glycine	23.3	21.8	22.1	21.1	21.5	3.8
Glutamic acid	59.6	60.3	63.9	64.0	67.6	5.1
Histidine	8.2	8.2	8.3	8.2	7.2	5.7
Isoleucine	16.2	15.6	16.3	16.2	16.6	2.2
Leucine	32.6	32.3	33.6	33.7	34.8	3.0
Lysine	25.6	25.2	25.6	23.9	24.8	2.8
Methionine	8.2	7.9	7.6	7.1	9.3	10.3
Methionine + cystine	12.8	12.2	12.2	10.7	14.0	9.6
Phenylalanine	16.3	16.2	17.2	17.2	18.1	4.6
Proline	23.6	23.2	24.6	24.2	25.5	3.7
Serine	18.3	18.1	19.2	19.4	20.6	5.2
Threonine	12.8	12.5	12.2	13.0	12.5	2.4
Tryptophan	2.3	2.5	2.2	2.8	2.9	12.0
Tyrosine	13.4	13.4	14.1	13.7	14.5	3.4
Valine	18.4	17.5	17.9	18.0	18.0	1.8

[1] Per cent substitution of fish meal (FM) for soy protein concentrate (SPC).
[2] Coefficient of variation (%) = (standard deviation/mean) × 100.

containing less FM and/or FO (Table 5). On the other hand, when FM was completely eliminated from experimental diets, concentrations of linoleic acid (18:2n-6, LOA) was the highest at 37.8 and 43.6 g kg^{-1} (diets with 100% substitution of FM for SPC). As a result, total concentration of PUFA was higher in diets with increased levels of soybean oil.

Experiment #3 incorporated KRL and WSM as feeding effectors into a basal diet (0_KrSq) with 50.0 g kg^{-1} FM, 77.5 g kg^{-1} SPC and 10 g kg^{-1} FO (Table 6). From this diet, three other isonitrogenous (380.3–385.9 g kg^{-1} crude protein) and isoenergetic (19.9–20.0 MJ kg^{-1}) diets were formulated to contain 5, 10 and 20 g kg^{-1} KRL and WSM (1 : 1 ratio) at the cost of PBM. Dietary levels of DL-methionine, L-lysine and magnesium sulphate were adjusted as higher levels of KRL and WSM were allowed to enter the formula.

Diets from all experiments were manufactured with laboratory equipment as described by Nunes *et al.* (2011). The proximate and amino acid composition of the experimental diets were determined following standard methods (AOAC 2002). Fatty acids analysis was carried out in a gas chromatograph according to the guidelines published by IAL (2005).

Data and statistical analyses

In all experiments, shrimp were individually weighed at stocking and harvest to determine their 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 (AFI, g of feed consumed per stocked shrimp) determined at a dry matter basis according to Nunes *et al.* (2006b).

Shrimp growth performance in experiments #1 and #3 was analysed through one-way ANOVA for completely randomized experiments. For experiment #2, the growth performance was analysed by two-way ANOVA arranged in a 4 × 2 factorial design. 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

Water salinity, pH and temperature in experiments #1, #2 and #3 fell within acceptable levels for the culture of *L. vannamei* (35 ± 1.6 g L^{-1}, 7.4 ± 0.3 and 28.6 ± 0.3 °C; 36 ± 0.9 g L^{-1}, 7.6 ± 0.1 and 28.4 ± 0.2 °C; 35 ± 0.9 g L^{-1}, 7.6 ± 0.3 and 28.6 ± 0.6 °C, respectively). No statistical differences in these parameters were observed between experimental treatments ($P > 0.05$, ANOVA).

In experiment #1, a higher replacement of FM by SPC led no differences in final shrimp survival (87.0 ± 5.9%), weekly shrimp growth rate (0.96 ± 0.09 g) and shrimp yield (783 ± 92 g m^{-2}) after 72 days of rearing (Table 7, $P > 0.05$, ANOVA). Also, complete replacement of FM for SPC resulted in no significant difference in shrimp body weight at harvest.

Final body weight, AFI and FCR for shrimp fed a diet containing no FM (13.84 ± 2.82 g, 33.5 ± 3.5 g $shrimp^{-1}$ and 3.06 ± 0.33, respectively) were not statistically different from those fed the control diet with 180 g kg^{-1} FM (14.34 ± 2.43 g, 28.2 ± 4.0 g $shrimp^{-1}$ and 3.04 ± 0.30, respectively; $P > 0.05$, Tukey's HSD). Only shrimp fed diet with 75% FM replacement displayed a lower final body weight (13.53 ± 2.71 g) than those fed the control diet without SPC ($P < 0.05$, Tukey's HSD).

In experiment #1, in diets containing SPC, there was an increase in AFI when 20 g kg^{-1} WSM was used in combination with higher levels of FO. The highest AFI was found when FM was used at 90 g kg^{-1} with 90 g kg^{-1}

Table 3 Ingredient and chemical composition of diets used in experiment #2

Ingredients	Diets/composition (g kg^{-1} of the diet, as is)							
Fish oil inclusion	20 g kg^{-1}				10 g kg^{-1}			
% Substitution FM/SPC[1]	0%	31%	61%	100%	0%	31%	61%	100%
FM, anchovy[2]	120.0	85.0	50.0	0.0	120.0	85.0	50.0	0.0
Soy protein concentrate[2]	0.0	38.5	77.5	133.4	0.0	38.4	77.5	133.2
Broken rice[2]	41.5	35.1	25.8	11.9	41.5	35.4	25.9	12.7
Fish oil[2]	20.0	20.0	20.0	20.0	10.0	10.0	10.0	10.0
Soybean oil[2]	10.5	13.3	18.0	25.1	20.4	23.0	27.9	34.5
Magnesium sulphate	1.1	0.7	0.7	0.8	1.2	0.7	0.7	0.8
L-lysine[3]	1.2	1.3	1.5	1.7	1.2	1.4	1.5	1.7
DL-methionine[2]	0.0	0.4	0.8	1.4	0.0	0.4	0.8	1.4
Others[4]	805.7	805.7	805.7	805.7	805.7	805.7	805.7	805.7
Proximate composition (g kg^{-1} of the diet, dry matter basis)								
Crude protein[5]	388	384	394	391	394	385	386	388
Crude fat[5]	100	90	95	97	93	89	94	98
Crude fibre[5]	15	17	17	19	18	16	13	17
Ash[5]	105	98	96	89	106	97	95	91
Nitrogen-free extract[6]	393	412	398	404	390	413	412	405
Gross energy[5] (MJ kg^{-1})	20	20	20	20	20	20	20	20

[1] Per cent substitution of fish meal (FM) for soy protein concentrate (SPC).
[2] Composition similar as in Table 1.
[3] ADM do Brasil Ltda. (Santos, São Paulo, Brazil). Guarantee levels per kg of product: 788.0 g kg^{-1} monohydrochloride L-lysine (minimum); 15.0 g kg^{-1} moisture (maximum).
[4] Others included: 330.0 g kg^{-1} of soybean meal[2], 250.0 g kg^{-1} of wheat flour[2], 150.0 g kg^{-1} of poultry by-product meal[2], 20.0 g kg^{-1} of vitamin–mineral premix[7], 15.0 g kg^{-1} of soybean lecithin[2], 13.0 g kg^{-1} of bicalcium phosphate, 10.0 g kg^{-1} of common salt, 10.0 g kg^{-1} of potassium chloride, 7.0 g kg^{-1} of synthetic binder[8], 0.7 g kg^{-1} of ascorbic acid polyphosphate[2].
[5] Analysed values.
[6] Calculated by difference (1000 − CP − fat − CF − ash).
[7] Rovimix Camarões. 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 mg; vitamin B1, 3000 mg; vitamin B2, 2500 mg; vitamin B6, 3500 mg; vitamin B12, 6 mg; nicotinic acid, 10 000 mg; pantothenic acid, 5000 mg; biotin, 100 mg; folic acid, 800 mg; vitamin C, 25 000 mg; cholin, 40 000 mg; inositol, 20 000 mg; iron 2000 mg; copper, 3500 mg; chelate copper, 15 000 mg; zinc, 10 500 mg; chelate zinc, 4500 mg; manganese, 4000 mg; selenium, 15 mg; chelate selenium, 15 mg; iodine, 150 mg; cobalt, 30 mg; chromium 80 mg; filler, 1000 g.
[8] Pegabind™. Bentoli Agrinutrition Inc. (Elgin, TX, USA). Synthetic pellet binder composed of urea formaldehyde.

SPC ($P < 0.05$, Tukey's HSD). Apparently, these higher AFI levels also raised FCR significantly, particularly in the same diet subjected to 50% FM replacement ($P < 0.05$, Tukey's HSD).

In experiment #2, final shrimp survival was above 89% for all diets regardless of the percentage of FM replaced by SPC ($P > 0.05$, ANOVA, Table 8). Similarly, inclusion of 10 or 20 g kg^{-1} FO had no significant effect on shrimp survival, weekly growth, yield, AFI and FCR ($P > 0.05$, ANOVA).

On the other hand, replacement of FM by SPC caused a significant effect on shrimp weekly growth, yield and AFI ($P < 0.05$, ANOVA, Table 8). Weekly shrimp growth fell at higher levels of FM replacement, particularly at 100% substitution with 10 or 20 g kg^{-1} FO and at 61% substitution with 10 g kg^{-1} FO ($P < 0.05$, Tukey's HSD). At 10 g kg^{-1} FO, there was also a detriment in shrimp yield and AFI when FM substitution levels were equal or above 61% ($P < 0.05$, ANOVA). Interestingly, differences in these parameters could not be detected when shrimp were fed diets containing 20 g kg^{-1} FO. FCR was not affected when FM was replaced by SPC or when FO was reduced from 20 to 10 g kg^{-1}.

Final shrimp body weight in experiment #2 reduced statistically as replacement of FM by SPC was increased ($P < 0.05$, ANOVA; Fig. 1). The reduction in shrimp body weight at harvest was more sensitive to FM replacement when 10 g kg^{-1} FO was used compared with 20 g kg^{-1}. At 20 g kg^{-1} FO, no differences could be observed in shrimp final body weight between diets with 31% FM replacement (8.9 ± 1.50 g) and no replacement at all (8.5 ± 1.41 g; $P > 0.05$, Tukey's HSD). Comparatively, at 10 g kg^{-1} FO, final shrimp body weight reduced progressively as higher levels of FM were replaced by SPC ($P < 0.05$, Tukey's HSD). Within each level of FM replacement, there was no

Table 4 Amino acid profile of diets used in experiment #2 (g kg^{-1} of the diet, dry matter basis; analysed)

Amino acid	Diets (g kg^{-1}, dry matter basis)								
	20 g kg^{-1}				10 g kg^{-1}				
Fish oil inclusion % Substitution FM/SPC[2]	0%	31%	61%	100%	0%	31%	61%	100%	CV[1] (%)
Alanine	19.2	18.8	18.8	18.2	19.4	18.6	17.0	16.3	6.0
Arginine	21.8	22.2	23.1	23.8	22.6	22.8	23.1	23.6	2.9
Aspartic acid	31.4	32.1	33.4	34.4	31.4	31.9	33.3	34.1	3.7
Cystine	4.1	4.9	4.3	5.1	5.0	5.2	4.3	4.3	9.5
Glycine	20.0	19.4	19.6	19.3	21.2	20.3	20.3	20.0	3.1
Glutamic acid	57.2	58.6	61.2	63.4	57.6	58.3	61.4	63.3	4.2
Histidine	7.5	7.5	7.8	7.9	7.1	7.3	7.9	8.1	4.5
Isoleucine	15.9	15.2	15.9	16.3	15.9	15.6	15.6	15.5	2.1
Leucine	29.2	29.5	30.4	30.8	30.6	30.4	30.6	30.9	2.0
Lysine	23.6	24.3	24.3	25.0	23.6	24.1	24.9	25.2	2.5
Methionine	7.6	7.5	7.7	7.7	7.2	7.4	7.8	7.9	3.0
Methionine + cystine	11.7	12.0	12.0	12.8	12.2	12.6	12.1	12.2	2.9
Phenylalanine	15.1	15.3	16.0	16.5	15.7	15.8	16.0	16.3	3.0
Proline	19.6	19.7	20.5	21.1	22.1	21.9	21.6	22.3	5.1
Serine	14.8	15.2	16.1	16.2	17.9	17.5	16.4	16.8	6.4
Threonine	11.2	11.3	11.6	11.4	11.6	11.6	11.6	11.3	1.5
Tryptophan	1.5	1.5	1.7	1.5	1.4	1.3	1.4	1.7	9.4
Tyrosine	12.0	12.3	12.4	12.7	12.2	12.4	12.7	12.7	2.1
Valine	17.6	17.3	17.5	17.7	18.3	17.7	17.2	16.7	2.6

[1] Coefficient of variation (%) = (standard deviation/mean) × 100.
[2] Per cent substitution of fish meal (FM) for soy protein concentrate (SPC).

statistically significant difference in final shrimp body weight ($P > 0.05$, Tukey's HSD).

In experiment #3, use of KRL in combination with WSM in diets with 50.0 g kg^{-1} FM had no effect over shrimp survival (96.4 ± 3.0%), AFI (8.1 ± 0.73 g $shrimp^{-1}$), weekly growth (0.86 ± 0.11 g), yield (246 ± 37 g m^{-2}) and FCR (2.33 ± 0.25). However, there was a significant increment in final shrimp body weight when a combination of 20 g kg^{-1} KRL and WSM (7.90 ± 1.25 g) was used compared with a diet deprived of these ingredients (7.52 ± 1.17 g; $P < 0.05$, Tukey's HSD, Fig. 2).

Discussion

The present work has shown that under a clear-water condition and in the absence of natural food, the dietary levels of FO in practical diets for *L. vannamei* have a significant impact on the amount of FM that can be replaced by SPC. Under these rearing conditions, complete replacement of FM by SPC led to no detrimental effects in shrimp growth performance only when minimum levels of FO were met.

It was observed that the quantitative need for FO in the diet increased as higher substitution levels of FM for SPC (FM/SBM) were carried out. In a diet containing 120 g kg^{-1} FM and 10 g kg^{-1} FO, for example, final shrimp body weight significantly reduced with progressive replacements of FM/SPC. Comparatively, as much as 31% replacement of FM/SPC was possible with 20 g kg^{-1} FO. Whenever dietary fat was adjusted by using FO as a lipid source (experiment #1), complete replacement of FM by SPC was achieved with no negative effect on shrimp growth.

In the present study, reductions in the content of LC-PUFA of experimental diets were consistent with lower inclusions of FM and FO. LC-PUFA fell to its lowest level in the diet (3.9 g kg^{-1} of the total fatty acids) when 10 g kg^{-1} FO and no FM were used. Conversely, as FM was replaced by SPC, the level of short-chain PUFA in the diets increased, regardless of the dietary FO level (i.e., 10 or 20 g kg^{-1}). As opposed to the LC-PUFA content, no apparent association appeared to exist between shrimp growth and dietary short-chain PUFA levels.

These results are corroborated with works done with *L. vannamei* by Lim *et al.* (1997) and González-Félix *et al.* (2003b). Lim *et al.* (1997) found that n-3 LC-PUFA had a better growth-promoting effect to *L. vannamei* than the short-chain PUFA 18:3n-3, due probably to the limited ability of shrimp to bioconvert fatty acids to polyenoic forms of longer chain length. González-Félix *et al.* (2003b) found that LC-PUFA had a greater nutritional value to

Table 5 Fatty acid composition (g kg^{-1} of the total dietary fatty acids, dry matter basis; analysed) of diets used in experiment #2

Fatty acid	Diets/composition (g kg^{-1} of the total dietary fatty acids)							
	20 g kg^{-1}				10 g kg^{-1}			
Fish oil inclusion % Substitution FM/SPC[1]	0%	31%	61%	100%	0%	31%	61%	100%
12:0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
14:0	3.3	2.6	2.3	2.0	1.5	1.5	1.4	1.0
15:0	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.1
16:0	17.3	15.2	17.6	17.4	19.3	18.6	16.5	15.9
17:0	0.5	0.5	0.4	0.4	0.4	0.3	0.3	0.3
18:0	4.4	3.8	4.6	4.5	5.6	5.4	4.5	4.2
16:1n-7	3.2	2.8	3.2	2.8	2.4	2.4	2.1	1.8
18:1n-9	19.0	18.3	22.6	22.6	22.2	22.5	23.5	24.1
18:2n-6	32.0	33.5	34.9	37.8	38.8	39.5	40.8	43.6
18:3n-3	5.2	4.9	5.2	5.4	5.5	5.5	5.0	5.1
20:3n-3	0.5	0.3	0.3	0.3	0.1	0.2	0.2	0.2
20:4n-6	0.5	0.5	0.3	0.3	0.1	0.1	0.2	0.1
20:5n-3	7.7	5.9	4.4	3.2	2.0	2.0	2.8	1.6
22:6n-3	5.81	4.8	3.7	3.0	1.8	1.8	2.4	2.2
SFA[2]	26.1	22.3	25.3	24.6	27.1	26.0	23.1	21.6
MUFA[3]	22.2	21.1	25.7	25.4	24.5	24.9	25.6	26.0
PUFA[4]	37.7	38.8	40.4	43.5	44.4	45.2	46.0	48.7
LC-PUFA[5]	14.0	11.2	8.4	6.5	4.0	3.9	5.3	3.9
Total n-3[6]	19.2	16.0	13.6	11.9	9.5	9.5	10.4	9.1
Total n-6[7]	32.5	34.0	35.2	38.1	38.9	39.6	41.0	43.5
n-3/n-6	0.59	0.47	0.39	0.31	0.24	0.24	0.25	0.21

LC-PUFA, long-chain polyunsaturated fatty acids.
[1] Per cent substitution of fish meal (FM) for soy protein concentrate (SPC).
[2] Saturated fatty acids: 12:0, 14:0, 15:0, 16:0, 17:0, 18:0.
[3] Monounsaturated fatty acids: 16:1, 18:1.
[4] PUFA: 18:2, 18:3, 20:3.
[5] LC-PUFA: 20:4, 20:5, 22:6.
[6] Total n-3: 18:3, 20:3, 20:5, 22:6.
[7] Total n-6: 18:2, 20:4.

juvenile *L. vannamei* than short-chain PUFA. Authors were not able to demonstrate that *L. vannamei* had any dietary requirement for C_{18} PUFAs. In diets for *Penaeus monodon*, Glencross & Smith (1999) found that maintaining an optimal balance between dietary linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids can improve shrimp growth. In a further work, an optimal ratio between n-3 and n-6 fatty acids was established at about 2.5 to 1 in diets for this species (Glencross *et al*. 2002). While penaeid shrimp appear to have no actual requirements for marine ingredients in their diets, FM and FO are both rich sources of LC-PUFA (NRC 1993, 2011; Turchini *et al*. 2009). As penaeids have a limited ability for synthesizing LC-PUFA (Kanazawa *et al*. 1979; Glencross & Smith 2001), a provision of marine oils in their diets is required until cheaper and more reliable sources to FO are identified.

Early work had already demonstrated that marine shrimp may desaturate 16:0, but elongation and desaturation products of 18 carbon fatty acids were not noted (Deering *et al*. 1997). Other lipid sources have been evaluated in marine shrimp diets with limited success. González-Félix *et al*. (2002a) conducted an 8-week feeding trial with *L. vannamei* fed five diets which contained 50 g kg^{-1} of coconut, soybean, linseed, peanut or menhaden oil in combination with 31 g kg^{-1} of a commercial lecithin. A diet without the tested lipid sources containing lecithin was used as a control. Authors reported that shrimp fed diets containing menhaden oil, with and without lecithin, showed higher final body weight and instantaneous growth rate (IGR) and lower FCR than the rest of the treatments. Patnaik *et al*. (2006) was able to successfully replace menhaden FO by heterotrophic algal sources in FM-free diets for *L. vannamei*. Shrimp were stocked at 30 animals m^{-2} and reared for 15 weeks in zero-water exchange circular tanks of 650 L. Authors used commercial spray-dried heterotrophic marine algal meals high in DHA (22:6n-3) and arachidonic (20:4n-6, ARA) acids to substitute FO. However, according to Turchini *et al*. (2009), alternative oil sources derived from unicellular algae, pelagic organisms or benthic invertebrates containing high amounts of n-3

Table 6 Ingredient and chemical composition of diets used in experiment #3

Ingredient	Diets[1]/composition (g kg^{-1} of the diet, as is)			
	0_KrSq	5_KrSq	10_KrSq	20_KrSq
Poultry by-product meal[2]	150.0	145.3	140.5	131.0
KRL[3]	0.0	2.5	5.0	10.0
WSM[2]	0.0	2.5	5.0	10.0
L-lysine[2]	1.5	1.2	0.9	0.3
DL-methionine[2]	0.8	0.7	0.6	0.4
Magnesium sulphate	0.7	0.9	1.0	1.3
Others[4]	846.9	846.9	846.9	846.9
Proximate composition (g kg^{-1} of the diet, dry matter basis)				
Crude protein[5]	386	380	381	381
Crude fat[5]	94	93	65	70
Crude fibre[5]	13	46	52	56
Ash[5]	95	95	95	96
Nitrogen-free extract[6]	412	385	406	397
Gross energy[5] (MJ kg^{-1})	20	20	20	20

Diets contained increasing dietary levels of krill meal (KRL) and whole squid meal (WSM), except control diet 0_KrSq.
[1] Treatment diets combined increased dietary levels of krill meal and WSM (1 : 1 ratio), 5.0 g kg^{-1} (0.5_KrSq), 10.0 g kg^{-1} (10_KrSq) and 20.0 g kg^{-1} (20_KrSq).
[2] Ingredient composition similar as in Tables 1 or 2*.
[3] QRILL™ meal, Aker Biomarine ASA (Oslo, Norway). 580 g kg^{-1} crude protein; 180 g kg^{-1} fat; 130 g kg^{-1} ash; 60 g kg^{-1} crude fibre; 71 g kg^{-1} moisture.
[4] Others included: 330.0 g kg^{-1} of soybean meal[2], 250.0 g kg^{-1} of wheat flour[2], 77.5 g kg^{-1} of soy protein concentrate[2], 50.0 g kg^{-1} of anchovy fish meal[2], 25.9 g kg^{-1} of broken rice[2], 27.9 g kg^{-1} of soybean oil[2], 10.0 g kg^{-1} of fish oil[2], 20.0 g kg^{-1} of vitamin–mineral premix*, 15.0 g kg^{-1} of soybean lecithin[2], 13.0 g kg^{-1} of bicalcium phosphate, 10 g kg^{-1} of common salt, 10.0 g kg^{-1} of potassium chloride, 7.0 g kg^{-1} of synthetic binder[2], 0.7 g kg^{-1} of ascorbic acid polyphosphate[2].
[5] Analysed values.
[6] Calculated by difference (100.0 − CP − fat − CF − ash).

Table 7 Final performance of *Litopenaeus vannamei* in experiment #1 stocked with 4.03 ± 0.73 g body weight in clear-water tanks (35 ± 1.6 g L^{-1} salinity, 7.4 ± 0.29 pH and 28.7 ± 0.7 °C temperature; n = 4350)

Performance Parameter	% Substitution of fish meal for soy protein concentrate						
	0%	25%	50%	75%	100%	Mean ± SE	*P*-value
Survival (%)	90.0 ± 2.6	89.5 ± 2.7	84.5 ± 2.4	83.5 ± 1.3	87.5 ± 3.4	87.0 ± 1.2	ns
Final body weight (g)	14.34 ± 0.18^{a}	14.09 ± 0.17^{ab}	14.03 ± 0.19^{ab}	13.53 ± 0.21^{b}	13.84 ± 0.21^{ab}	–	0.044
Weekly growth (g)	1.00 ± 0.05	1.00 ± 0.02	0.97 ± 0.02	0.92 ± 0.04	0.93 ± 0.06	0.96 ± 0.02	ns
Yield (g m^{-2})	615 ± 39	617 ± 22	552 ± 38	507 ± 31	553 ± 63	569 ± 19	ns
AFI (g shrimp^{-1})	28.2 ± 1.8^{a}	33.8 ± 2.7^{ab}	38.7 ± 2.5^{b}	33.5 ± 1.5^{ab}	33.5 ± 1.6^{ab}	–	0.032
FCR	3.04 ± 0.30^{a}	2.91 ± 0.52^{ab}	3.59 ± 0.65^{b}	3.28 ± 0.36^{b}	3.06 ± 0.33^{ab}	–	0.006

Values are means (±standard error) of five rearing tanks, except final shrimp body weight which refers to the means of all harvested animals
Common letters in rows denote non-significant differences among experimental diets at the $\alpha = 0.05$ level by Tukey's HSD multiple range test.
ns, not statistically significant ($P > 0.05$); AFI, apparent feed intake; FCR, food conversion ratio.

LC-PUFA, appear promising, but unfeasible for use in aquafeeds in the short to medium term.

In the present study, the LC-PUFA threshold prior to loss in *L. vannamei* growth performance was detected at 11.2 g kg^{-1} of the total fatty acids with an n-3/n-6 ratio of 0.45. At these levels, EPA (20:5n-3) and DHA acids achieved 5.9 and 4.8 g kg^{-1} of the total fatty acids content, respectively. Glencross & Smith (2001) found that in diets for the tiger shrimp, *P. monodon*, both EPA and DHA acids have a growth-promoting effect. They reported a better shrimp growth when combinations of EPA and DHA were used; the optimal combination being EPA 4 g kg^{-1}

Table 8 Final performance of *Litopenaeus vannamei* in experiment #2 stocked with 2.02 ± 0.51 g body weight in clear-water tanks (35 ± 0.9 g L^{-1} salinity, 7.6 ± 0.29 pH and 28.5 ± 0.5 °C temperature; n = 3984)

Performance parameter	% Substitution FM/SPC[1]	Dietary fish oil (FO) level	
		20 g kg^{-1}	10 g kg^{-1}
Survival (%)	0	91.3 ± 2.3	94.2 ± 0.8
	31	90.0 ± 2.0	94.2 ± 1.2
	61	93.3 ± 1.7	89.6 ± 2.1
	100	94.6 ± 1.8	91.3 ± 2.2
Weekly growth (g)	0	0.67 ± 0.02^{a}	0.72 ± 0.03^{a}
	31	0.64 ± 0.01^{ab}	0.68 ± 0.03^{ab}
	61	0.61 ± 0.04^{ab}	0.58 ± 0.02^{bc}
	100	0.56 ± 0.03^{b}	0.52 ± 0.02^{c}
Yield (g m^{-2})	0	533 ± 20	587 ± 23^{a}
	31	505 ± 6	561 ± 18^{a}
	61	503 ± 19	470 ± 22^{b}
	100	488 ± 25	441 ± 24^{b}
Apparent feed intake (AFI, g $shrimp^{-1}$)	0	13.3 ± 0.4	14.4 ± 0.2^{a}
	31	13.1 ± 0.1	14.1 ± 0.4^{a}
	61	12.1 ± 0.4	12.6 ± 0.5^{ab}
	100	11.7 ± 0.7	11.6 ± 0.8^{b}
FCR	0	1.77 ± 0.09	1.73 ± 0.05
	31	1.83 ± 0.03	1.77 ± 0.04
	61	1.69 ± 0.05	1.89 ± 0.09
	100	1.67 ± 0.03	1.85 ± 0.07

Two-way ANOVA	Survival	Growth	Yield	AFI	FCR
% Substitution FM/SPC	ns	<0.0001	<0.0001	<0.0001	ns
FO inclusion	ns	ns	ns	ns	ns
% Substitution × FO[1]	ns	ns	0.019	ns	ns

Values are means (±SE) of six rearing tanks.
For each FO level, different letters in columns denote statistically significant differences among diets at the $\alpha = 0.05$ level by Tukey's HSD multiple range test. Absence of letters in columns indicate non-significant difference.
ns, not statistically significant ($P > 0.05$); FCR, food conversion ratio.
[1] Per cent substitution of fish meal (FM) for soy protein concentrate (SPC); FO, fish oil.

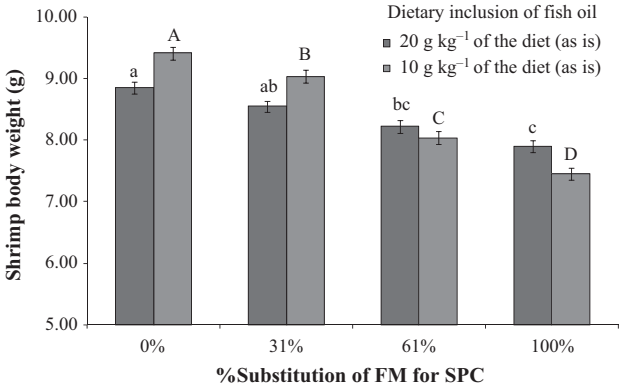

Figure 1 Final body weight of *Litopenaeus vannamei* fed diets at two dietary levels of fish oil (FO), 10 and 20 g kg^{-1}, and increasing substitution levels of fish meal (FM) for soy protein concentrate (SPC). Columns with the same lowercase and capital letters indicate statistically significant difference at the $\alpha = 0.05$ level by Tukey's HSD multiple range test among diets with a similar level of FO. Columns are means (±standard error) of all harvested shrimp.

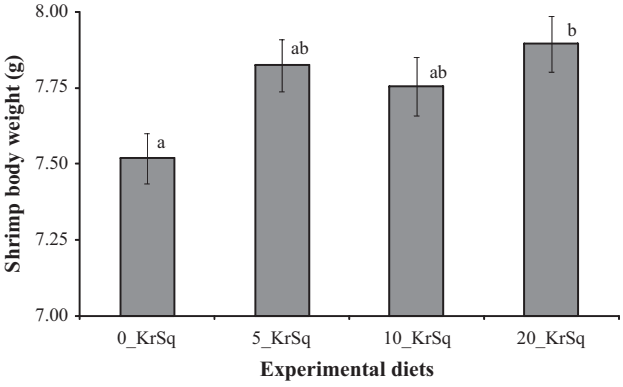

Figure 2 Final body weight of *Litopenaeus vannamei* fed diets with 50 g kg^{-1} fish meal and increasing levels of krill meal and whole squid meal in clear-water tanks (35 ± 0.9 g L^{-1} salinity, 7.6 ± 0.30 pH and 28.6 ± 0.6 °C temperature; n = 3197). Shrimp were stocked with 3.95 ± 0.67 g (n = 220) body weight. Columns with the same letters indicate statistically significant difference at the $\alpha = 0.05$ level by Tukey's HSD multiple range test. Columns are means (±standard error) of all harvested shrimp.

and DHA 4 g kg^{-1} of the total fatty acids. Similarly, in a 6-week study with *L. vannamei*, González-Félix *et al.* (2002b) reported that IGR of shrimp was enhanced by the addition of either DHA or n-3 LC-PUFA at 2.5 g kg^{-1} of the diet, although no further benefit was detected at 5.0 g kg^{-1}. In another study designed to evaluate nutritional value of dietary linoleic (18:2n-6, LOA) and LNA (18:3n-3) acids for *L. vannamei*, González-Félix *et al.* (2003a) found a higher final weight and weight gain for shrimp fed a diet containing 5.0 g kg^{-1} of a mixture of n-3 LC-PUFA.

Growth experiments with penaeids have shown that the lower levels of methionine found in proteins derived from soybeans must be overcome by supplementation with synthetic methionine (Swick *et al.* 1995; Alam *et al.* 2005; Forster & Dominy 2006; Huai *et al.* 2010; Browdy *et al.* in press) or by the addition of natural sources of intact methionine, such as corn gluten meal (Amaya *et al.* 2007a,b;

Sookying & Davis 2011), which is naturally rich in this amino acid (NRC 1993, 2011). The inclusion of corn gluten meal as a source of intact methionine is a less widespread formulation approach as it has a low apparent protein digestibility for *L. vannamei* (59.1% according to Lemos *et al.* 2009).

In the present work, even with an adequate supply of FO, shrimp performance was negatively affected when dietary methionine levels reached 7.1 g kg^{-1} (diet with 75% substitution of FM/SPC in experiment #1), 22% below the mean of 8.2 ± 1.1 g kg^{-1} found for other diets. Paripatananont *et al.* (2001) working with *P. monodon* concluded that as much as 50% in a diet containing 350 g kg^{-1} FM could be replaced by SPC. Further substitution levels led to a significant decrease on shrimp feed intake and body weight gains. Authors increased the inclusion levels of FO and soybean oil (1 : 1 ratio) as FM was withdrawn from the diets. However, in their study, diets were not supplemented with synthetic methionine that could have restrained shrimp growth.

While more recent studies have been able to demonstrate that it is possible to fully replace FM by soybean meal (SBM) and other cheaper plant protein sources, formulas have usually relied on high levels of FO. Amaya *et al.* (2007a) fed diets to *L. vannamei* in an outdoor system that contained 160 g kg^{-1} of PBM and progressive replacements of FM by SBM. Lipid levels in the diets were adjusted with menhaden FO included from 39.6 g kg^{-1} with 90 g kg^{-1} FM to a maximum of 47.2 g kg^{-1} in diets without FM. Authors were able to fully replace FM by SBM without any deleterious effect on shrimp growth performance. More recently, Sookying & Davis (2011) conducted an outdoor pond and tank study with *L. vannamei* at stocking densities of 35 and 30 animals m^{-2}, respectively. Shrimp were fed four diets that contained high levels of SBM (from 537.1 to 580.0 g kg^{-1}) as the primary protein source with the inclusion of either 100 g kg^{-1} PBM, FM, distiller's dried grains with solubles, or ground pea meal. Diets contained from 48.3 to as much as 58.2 g kg^{-1} FO. Authors found no negative impact on shrimp growth performance when fed these practical diets.

In comparison to the work of Amaya *et al.* (2007a) and Sookying & Davis (2011), the present work challenged the dietary levels of FO, adopted higher stocking densities (70 shrimp m^{-2}) and used a controlled clear-water rearing system. It appears that under a lower stocking density and with continuous access to naturally occurring food items, shrimp requirement for marine lipid sources is lower than what has been determined in the present work. González-Félix *et al.* (2010) successfully replaced up to 90% of menhaden FO (lowest inclusion of 4.6 g kg^{-1} of the diet) using a variety of lipid sources (mainly soybean and linseed oils) in a plant-based diet for *L. vannamei*. Shrimp were reared for 58 days under 26 animals $tank^{-1}$ in an outdoor system where they had access to natural foods.

Previous work carried out with crustaceans has found that soy products, especially soybean meal, are ingredients with lower palatability proprieties than FM (Floreto *et al.* 2000; Nunes *et al.* 2006a; Alvarez *et al.* 2007). Alvarez *et al.* (2007) working with *Litopenaeus schmitti* observed a significant increase in FCR and a decline in protein efficiency ratio and final body weight when shrimp were fed a diet with 540 g kg^{-1} SBM and no FM. Authors associated the loss in performance to a poor palatability of the FM-free diet. In the present work, WSM was incorporated into SPC-based diets to stimulate shrimp appetite and mask an expected lower palatability (experiment #1). The addition of 20 g kg^{-1} WSM in the diets with SPC was able to induce shrimp feed intake at levels higher than the control diet with 180 g kg^{-1} FM, but also led to a poorer FCR at 50% or higher substitution of FM/SPC. However, there was no consistency between higher inclusions of SPC and loss in shrimp feed efficiency.

In experiment #2, when WSM was pulled out from diets containing SPC, there was a trend towards a progressive reduction in AFI, particularly when 10 g kg^{-1} FO was used. In this case, two-way ANOVA analyses revealed that FCR was unaffected by the percentage substitution of FM/SPC, although a statistically significant effect was found for AFI. It seems the detrimental FCR found in experiment #1 for SPC-based diets was driven by an increased shrimp feed intake, not a lower energy or protein digestibility of SPC. Cruz-Suárez *et al.* (2009) reported both high energy and protein digestibility values for a commercial SPC obtained in Mexico (85.1 ± 3.9 and 93.0 ± 1.5%, respectively) the latter being higher than the apparent digestibility values found for the two sources of Anchovy FM (87.9 ± 0.7 and 88.5 ± 2.4%; Lemos *et al.* 2009).

Experiment #3 revealed that a combination of WSM and KRL at dietary levels starting at 5 g kg^{-1} can enhance shrimp final weight in a SPC-based diet containing only 50 g kg^{-1} FM. A more pronounced effect on final shrimp weight was found when incorporation of both feeding effectors summed up to 20 g kg^{-1} of the diet. Enhancement of shrimp body weight was most likely driven by an increased feed intake and higher nutrient delivery. However, since in this experiment, rearing period was reduced to 30 days, differences in AFI could not be detected among

experimental diets. Recently, a similar effect was found by Suresh *et al*. (2011) in diets containing high levels of PBM formulated for the blue shrimp, *Litopenaeus stylirostris*. Shrimp were fed for 6 weeks with diets containing 200 g kg^{-1} PBM, no FM and 30 g kg^{-1} of blood meal, hydrolysed feather meal, anchovy FM, fish hydrolysate, squid liver meal (SLM) and KRL. Authors found that while attractability of the feeds was improved by SLM and KRL, palatability and growth were improved only by KRL. These results are consistent with the present study which also showed an improved feed intake of SPC-based diets containing WSM and a higher final shrimp weight for SPC-based diets containing both KRL and WSM.

Conclusions

Results from this work demonstrated that a multi-nutrient formulation approach needs to be taken to effectively replace FM by SPC in diets for *L. vannnamei*. The amount of FO in the diets, and ultimately their dietary n-3 LC-PUFA content, was found to have a major affect on the per cent replacement of FM by SPC that can be achieved without adverse effects to shrimp growth performance. However, a minimum provision of n-3 LC-PUFA in the diet alone did not result into a successful replacement of FM when other nutrient-restraint aspects of SPC-based diets were neglected. These included supplementing diets containing high levels of SPC with essential amino acids, chemoattractants and feeding stimulants.

While the global supply of FM and FO for use in aquafeeds is both at stake owing to an increased growth in direct human consumption (FAO 2010), marine shrimp feeds are quantitatively much more dependent on the former (Tacon & Metian 2008). Therefore, as the use of SPC becomes more widespread in aquafeeds and market prices more economical, the cost for supplementing diets with n-3 LC-PUFA lipid sources, synthetic amino acids and feeding stimulants will most likely be offset by the lower or no dependence at all on FM in shrimp formulas.

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