Latin American Journal of Aquatic Research, 48(3): 396-405, 2020
DOI: 10.3856/vol48-issue3-fulltext-2361

Research Article

Production of *Penaeus vannamei* in low salinity, using diets formulated with different protein sources and percentages

**Juan Carlos Gil-Núñez[1], Luis Rafael Martínez-Córdova[2], Rosalía Servín-Villegas[3]
Francisco Javier Magallon-Barajas[3], Rafael Apolinar Bórquez-López[4]
Jose Reyes Gonzalez-Galaviz[5] & Ramón Casillas-Hernández[4]**

[1]Departamento de Biotecnología y Ciencias Alimentarias del Instituto Tecnológico de Sonora, Obregón Sonora, México
[2]Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora, Hermosillo Sonora, México
[3]Centro de Investigaciones Biológicas del Noroeste, S.C., Instituto Politécnico Nacional La Paz, México
[4]Departamento de Ciencias Agropecuarias y Veterinarias del Instituto Tecnológico de Sonora, Obregón Sonora, México
[5]CONACYT - Instituto Tecnológico de Sonora, Obregón, Sonora, México
Corresponding author: Ramón Casillas-Hernández (ramon.casillas@itson.edu.mx)

ABSTRACT. The present study was focused on assessing the effect of diets formulated with different sources and levels of protein on the production response of white leg shrimp *Penaeus vannamei* farmed at low salinity (3 g L^{-1}). The protein sources were: soy meal (SM) and fish meal (FM), included at three levels: low (22-25%), medium (32-35%), and high (41-45%). A bioassay of 49 days was done in experimental tanks of 100 L (three tanks per treatment). Juvenile *P. vannamei* having a mean weight of 1.41 ± 0.30 g were stocked at a density of 100 ind m^{-3}. Shrimp fed diets formulated with FM recorded significantly higher weight gain, specific growth rate (SGR), and protein assimilation efficiency rate (PER). Additionally, it showed a higher concentration of essential amino acids like methionine and threonine, and higher ash, calcium, iodine, phosphorous, and sodium content, which could favor the growth and survival of shrimp. Regression analysis showed that the optimum protein levels for diets formulated with SM and FM were 34.8 and 29.3%, respectively. These findings may help shrimp farmers to implement better feeding strategies for *P. vannamei* farmed at low salinity and on the use of alternative ingredients to substitute fish meal in the formulated feed.

Keywords: *Penaeus vannamei*; low salinity; soybean meal; replacement; diet; aquaculture

INTRODUCTION

Farming of white leg shrimp *Penaeus vannamei* at low salinity is a practice becoming popular in diverse regions of the world. The species ability to thrive in a salinity range from 0.5 to 45 g L^{-1}, in waters with an adequate ionic balance, make it a viable alternative for culture in diverse aquatic environments (González-Félix *et al*., 2017), since it has been farmed in waters from wells as well as in salinized land unable for agriculture (Páez-Osuna & Valencia-Castañeda, 2013; Jarwar, 2015). Moreover, due to the negative effect that marine aquaculture has had as a result of various viral diseases, the inland farming of marine organisms has become a viable alternative (Fierro *et al*., 2018). However, the culture at low salinities faces some challenges, some of them related to nutrition and environmental sustainability (Chen *et al*., 2015).

The main aquafeeds protein ingredient is fish meal (FM), mainly because of its high digestibility, palatability, and balanced amino acid profile (NRC, 2011; Bauer *et al*., 2012; Huang *et al*., 2018). However, the increasing price and decreasing FM availability are two important problems for its use (Katya *et al*., 2016;

Corresponding editor: Fernando Vega

Ayisi *et al*., 2017). Many different studies have been conducted to substitute FM in aquafeeds using some other protein sources such as fly meal, pea paste, cotton seeds, and poultry subproducts (Lim, 1996, 1997; Liu *et al.*, 2012; Luo *et al.*, 2012; Cabanillas-Beltrán *et al.*, 2013; Carvalho *et al.*, 2016; Cummins *et al*., 2017; Panini *et al*., 2017a,b). Soy meal (SM) has shown to be a good substitute for FM in diets for *P. vannamei* due to its high content of crude protein and good amino acid profile, as well as its low cost as compared to FM (Jatobá *et al*., 2017). There are many studies on the replacement of FM by SM in shrimp *P. vannamei* both in marine and low salinity environments, reporting the performance, protein efficiency ratio, daily feeding intake and mineral supplements (Xie *et al*., 2016; Huang *et al*., 2017; Jatobá *et al*., 2017; Moreno-Arias *et al*., 2018). However, the effect of the protein level of different sources has not been sufficiently explored in the culture at low salinity. For that reason, this study was focused on evaluating some sources with varying levels of protein on the productive performance of *P. vannamei* farmed at low salinities and found the optimal level for that condition.

MATERIALS AND METHODS

Diets preparation and chemical analysis

After analyzing the proximate chemical composition of the four meals (PIASA; La Paz, BCS, México) used as ingredients (fish meal: FM, soy meal: SM, soy protein concentrate: SPC, and wheat flour; WF), six isocaloric and isolipidic diets were prepared (Table 1). The formulation was done by using algorisms developed by Calderón (2007). Fish meal and SM were used as the main protein sources, and were included at three levels in the respective diets: low (L, 22-25%), medium (M, 32-35%) and high (H, 41-45%), resulting in the six experimental diets: FM_L, FM_M, FM_H, SM_L, SM_M, SM_H. In the six diets, fish oil was used as a lipid source. Diets based on animal protein were prepared with a mix of 36.4% FM, 36.4% SM, and 27.1% WM. Diets based on vegetal protein, were prepared with 45.0% soy protein concentrate, 30.0% SM, 20.0% WM and 2.4 to 4.4% FM as attractant (Table 2). The analyzes of amino acid and elemental components were done by HPLC accordingly to the method suggested by Vázquez-Ortiz *et al*. (1995).

Obtaining and acclimation of experimental organisms

Shrimp postlarvae of *Penaeus vannamei* were obtained from Larvas Génesis S.A de C.V (Sonora, México). They were acclimated in plastic tanks under controlled conditions of temperature (28 ± 0.5°C), salinity (35) and dissolved oxygen (DO) (≥4 mg L^{-1}), feeding them three times a day (08:00, 13:00 and 18:00 h), at satiations with a commercial feed (35% CP, crude proteins; 7% lipids). Feces and unconsumed feed were discarded by siphoning. Ten percent of the water was exchanged every 24 h. When shrimp reached 1.0-1.5 g bodyweight, the acclimation to the salinity process (from 35 to 3) was initiated, according to suggested by Van Wyk (1999).

Experimental design

A bioassay was conducted over 49 days to evaluate the experimental diets. A completely randomized experimental design with three replicate per treatment was performed. The experimental units consisted of 21 tanks of 200 L, three for each of the six diets, and three for the control. Each unit was stocked with 10 juveniles (100 ind m^{-3}; 1.41 ± 0.30 g) which were maintained under relatively controlled conditions (temperature: 28 ± 0.5°C; pH: 8.3-8.4; DO: 6.40 ± 0.72 mg L^{-1}; salinity: 3; photoperiod: 12:12 h).

Three times during the trial (days 1, 28 and 49), the concentrations of K^+, Mg^{++}, Ca^{++}, Na^+ and Cl^-, were measured by spectrophotometry (Spectroquant 300 Merck) with precisions around, 2.6, 1.6, 1.9, 2.4, and 2.1%, respectively. A water exchange of around 10% d^{-1} was applied. Shrimp were fed at satiation three times a day (08:00, 13:00, and 18:00 h), initiating with 10% of total shrimp biomass, adjusting it weekly. Unconsumed feed was removed and stored at 20°C. At the end of the trial, the production responses of shrimp: final weight (FW), % of weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival (S) were estimated, accordingly to Peña-Rodríguez *et al.* (2017):

FW = Σ final individual weight / final number of shrimps

WG = 100 × (final weight - initial weight) / initial weight

SGR (% d^{-1}) = 100 × (ln final weight - ln initial weight / days of culture)

FCR = consumed feed / (final weight - initial weight)

PER = (final weight - initial weight)/(consumed feed × protein concentration)

S = (number of harvested shrimp/number of stocked shrimp) × 100

Statistical analysis

Production parameters were analyzed by a two-way analysis of variance (after homoscedasticity and normality test) set to a confidence level of $P < 0.05$. The analysis was performed in the software Statgraphics XVI.

Table 1. Results of bromatological analysis of each of the flours used in the preparation of experimental diets.

Analysis	Fish meal	Soybean meal	Soy protein concentrate	Wheat flour
Moisture (%)	6.59	8.87	8.27	10.45
Crude protein (%)	72.95	50.95	64.70	13.38
Crude fat (%)	6.38	1.17	1.13	0.40
Ash (%)	16.98	7.16	3.54	0.80
Crude fibre (%)	0.13	2.80	4.33	0.30
Nitrogen-free extract (%)	3.56	38.05	26.30	85.12

Table 2. Formulation of the six experimental diets for *Penaeus vannamei*, based in vegetal and animal protein (g kg^{-1} diet). SM: soy meal, FM: fish meal. SM_L: diet with 25% protein; SM_M: diet with 35% protein; SM_H: diet with 45% protein (based on mixture of vegetal proteins); FM_L: diet with 22% protein; FM_M: diet with 32% protein; FM_H: diet with 41% protein (based on mixture of vegetal and animal protein). a) Whole sardine meal (72.95% crude protein, 6.38% lipid)*, b) soy protein concentrate (SPC) (64.70% crude protein, 1.13% lipid)*, c) soybean meal (50.95% crude protein, 1.17% lipid)*, d) wheat flour (13.38% crude protein, 0.4% lipid)*, e) corn starch (0.3% crude protein, 0.1% lipid), Maizena®, f) fish oil*, g) soybean lecithin*, h) mineral premix*, i) vitamin premix*, j) alginic acid**, k) ash**, l) cellulose, m) antioxidant. *PIASA (La Paz, BCS, México), **Sigma-Aldrich Corp. 180947A2158 (St. Louis, MO, USA).

	Treatment					
	SM_L	SM_M	SM_H	FM_L	FM_M	FM_H
Fish meal[a]	24.83	34.77	44.70	186.39	247.56	325.55
Soy protein concentrate[b]	223.50	312.92	402.36	0.00	0.00	0.00
Soybean meal[c]	149.00	208.62	268.24	186.39	247.56	325.55
Wheat flour[d]	99.33	139.08	178.83	138.86	184.43	242.53
Corn starch[e]	371.19	190.08	8.95	356.19	168.96	0.00
Fish oil[f]	28.72	26.22	23.72	19.39	64.23	37.30
Soy lecithin[g]	36.20	36.20	36.21	35.21	33.40	34.17
Mineral premix[h]	9.24	9.25	9.25	8.99	8.53	8.72
Vitamin premix[i]	9.24	9.25	9.25	8.99	8.53	8.72
Alginic acid[j]	18.49	18.49	18.49	17.98	17.06	17.45
Ash[k]	18.88	9.44	0.00	36.89	17.49	0.00
Cellulose[l]	11.34	5.67	0.00	4.70	2.23	0.00
Antioxidant[m]	0.01	0.01	0.01	0.01	0.01	0.01

The optimal requirements of protein were estimated for the maximum WG by using the software MATLAB with the curve adjustment tools (Mathworks, 2012), using as independent variables, the three levels of each protein source. The selection criteria for curve adjusting were based on the determination coefficient (R^2). From the second order-polynomial regression, the optimal level supporting the response of maximum WG was calculated (Li *et al.*, 2016; Bórquez-Lopez *et al.*, 2018).

RESULTS

Effect of a fish meal replacement on the proximate chemical composition, amino acid profile and elemental components of the diets

The proximate chemical composition and amino acid profile of diets are shown in Table 3. Ash content was higher in diets based on FM. The content of nitrogen-free extract (NFE) decreased as the percentage of protein supplemented increased, independently of the source of protein used. The energy content was similar among diets. Methionine and threonine content was a little lower in diets based on vegetal protein. Some elemental components such as Ca, I, P and Na, were found at higher proportion in diets based on FM.

Water quality parameters

Table 4 shows the water quality parameters, including the concentration of specific ions. Mean temperature was 28.2 ± 0.59°C, mean DO 6.4 ± 0.72 mg L^{-1}, mean pH 8.37 ± 0.06 and mean salinity 3.19 ± 0.15. The Mg^{++}, Na^+, and Cl^- concentrations remained under the optimal values required. The Na^+/K^+ rate ranged from 21:1 to 23:1 in the treatments.

Table 3. Proximate composition (g kg^{-1} fed), amino acid profile (% / %CP), and elementals compounds (ppm) of the six experimental diets for low salinity shrimp *Penaeus vannamei* elaborated with a different protein source (vegetal and animal). SM: soy meal, FM: fish meal. SM_L: diet with 25% protein; SM_M: diet with 35% protein; SM_H: diet with 45% protein (based on mixture of vegetal proteins); FM_L: diet with 22% protein; FM_M: diet with 32% protein; FM_H: diet with 41% protein (based on mixture of vegetal and animal protein).

	SM_L	SM_M	SM_H	FM_L	FM_M	FM_H
Proximate composition (g kg$^{-1}{}_{ms}$, except moisture)						
Moisture	46.0 ± 2.2	12.2 ± 0.8	18.8 ± 0.3	47.6 ± 1.6	28.5± 0.5	39.8 ± 0.3
Crude protein	256.7 ± 0.3	356.3 ± 0.7	444.0 ± 2.0	227.3 ± 1.1	321.2 ± 1.2	405.2 ± 2.5
Crude fat	63.8 ± 1.0	66.5 ± 0.0	65.3 ± 0.6	58.0 ± 0.9	58.4 ± 0.5	58.6 ± 1.9
Crude fiber	12.6 ± 0.6	11.7 ± 0.6	13.3 ± 0.6	2.3 ± 0.6	2.3 ± 0.6	7.0 ± 1.0
Ash	55.3 ± 0.4	55.9 ± 0.3	56.1 ± 0.3	103.8 ± 0.4	108.6 ± 0.3	115.1 ± 0.3
NFE	611.6	509.6	421.3	608.6	509.5	414.1
Gross energy (kcal)	4.48 ± 0.002	4.64 ± 0.004	4.79 ± 0.001	4.20 ± 0.006	4.33 ± 0.002	4.41 ± 0.002
Amino acid profile (% / %CP)						
Essential amino acid						
Arginine	3.50	3.56	3.40	3.00	3.37	3.26
Phenylalanine	5.75	5.41	5.06	5.22	4.83	4.86
Histidine	5.20	5.55	7.18	5.68	6.89	7.64
Isoleucine	3.54	3.86	3.34	3.50	3.02	3.09
Leucine	5.94	7.04	6.09	5.78	5.33	5.15
Lysine	0.61	1.06	1.26	1.13	1.45	0.85
Methionine	2.70	2.07	1.81	2.84	2.54	2.17
Threonine	9.06	14.07	16.32	16.61	20.85	16.64
Valine	4.07	4.26	4.10	4.71	4.01	4.34
Nonessential amino acid						
Alanine	2.48	3.60	3.54	3.69	4.38	3.87
Aspartic acid	5.45	5.17	5.94	4.89	6.23	5.99
Glutamic acid	18.42	15.56	17.44	13.68	16.41	15.21
Glycine	3.42	3.89	4.06	2.80	3.59	3.30
Serine	2.60	2.90	3.15	2.17	2.36	2.49
Tryptophan	11.90	5.56	6.33	9.48	7.49	8.25
Elementals compounds						
Ca	6283.6	6400.1	7412.2	14337.0	17700.5	26739.3
Cu	50.8	50.6	53.8	45.6	53.0	59.8
Fe	418.9	413.5	597.5	613.8	417.5	970.3
K	4143.3	5922.3	6453.1	3886.0	6289.4	6328.6
Mg	1227.5	1444.7	1862.5	1375.9	1763.4	2696.4
Mn	22.6	26.9	35.0	23.1	26.4	41.0
Na	3281.8	3077.2	2822.8	3717.8	4792.5	4310.3
P	4876.1	5863.3	7014.5	8312.6	10406.4	15861.7
S	807.7	929.1	1185.1	725.5	891.0	1336.5
Si	37.8	20.9	19.0	33.8	31.9	26.7
Zn	129.8	120.6	132.0	138.2	139.8	183.3
N	32550.0	94150.0	57050.0	35350.0	43750.0	51450.0

Effect of experimental diets, on the production response and feed use efficiency

Table 5 presents the production response parameters, and the feed use efficiency of shrimp fed the experimental diets and the control. The weight gain rate ranged from 219 to 389%, the SGR from 1.01 to 1.41%, the survival from 60.0 to 93.3%, the FCR from 1.64 to 2.15. The PER varied from 0.92 to 2.83. The WG showed significant differences among diets FM_L and SM_L ($P < 0.05$).

Effect of experimental diets on the optimal protein level and maximum weight gain

Table 6 shows the optimal protein level for the two sources of protein used. For diets based on FM, the optimal level was 29.3%, while for those based on SM, it was 34.8%.

Table 4. Water quality results (mean ± standard deviation) of low salinity shrimp (*Penaeus vannamei*) cultured for 49 days fed six experimental diets and one commercial diet (n = 3). SM: soy meal, FM: fish meal. SM: soy meal, FM: fish meal. SM_L: diet with 25% protein; SM_M: diet with 35% protein; SM_H: diet with 45% protein (based on mixture of vegetal proteins); FM_L: diet with 22% protein; FM_M: diet with 32% protein; FM_H: diet with 41% protein (based on mixture of vegetal and animal protein); DC: Diet Control (commercial feed: 35% crude proteins; 7% lipids). *(Paez-Osuna & Valencia, 2013; Hongyu *et al.*, 2014).

Water quality parameter	Treatment							*Optimal value
	SM_L	SM_M	SM_H	FM_L	FM_M	FM_H	DC	
Temperature (°C)	28.2 ± 0.58	28.3 ± 0.66	28.7 ± 0.58	28.2 ± 0.59	28.2 ± 0.57	28.2 ± 0.57	28.2 ± 0.59	24 - 32
Dissolved oxygen (mg L^{-1})	6.7 ± 0.77	6.4 ± 0.74	6.3 ± 0.71	6.4 ± 0.72	6.4 ± 0.67	6.4 ± 0.75	6.4 ± 0.68	≥ 4.0
pH	8.4 ± 0.06	8.4 ± 0.06	8.4 ± 0.05	8.4 ± 0.06	8.4 ± 0.08	8.4 ± 0.07	8.4 ± 0.07	7 - 9
Salinity	3.2 ± 0.19	3.2 ± 0.15	3.2 ± 0.20	3.2 ± 0.09	3.2 ± 0.11	3.2 ± 0.15	3.2 ± 0.15	0.5 - 40.0
Potassium (mg L^{-1})	38.3 ± 5.36	37.3 ± 4.44	39.9 ± 4.53	37.8 ± 5.15	38.3 ± 3.92	38.7 ± 3.01	35.9 ± 5.71	33.9
Magnesium (mg L^{-1})	66.9 ± 15.8	52.6 ± 17.7	67.1 ± 17.4	62.3 ± 12.0	64.1 ± 12.9	65.2 ±14.7	51.0 ±11.8	109.5
Calcium (mg L^{-1})	53.9 ± 12.9	49.5 ± 8.4	58.2 ± 13.0	52.8 ± 12.4	55.6 ± 14.5	53.6 ± 11.2	45.7 ± 9.3	35.1
Sodium (mg L^{-1})	868.9 ± 245.6	771.1 ± 196.5	906.7 ± 201.1	818.9 ± 210.3	888.9 ± 148.8	873.3 ± 176.1	644.4 ± 316.7	919.8
Chloride (mg L^{-1})	1530.0 ± 228.6	1468.9 ± 264.8	1657.8 ± 255.5	1576.7 ± 349.6	1674.4 ± 247.6	1588.9 ± 184.8	1422.2 ± 431.5	1650.9
Relation Na^+/K^+	23:1	21:1	23.1	22:1	23:1	23:1	18:1	23:1

As a relatively recent activity in México, farming marine shrimp at low salinity requires yet much investigation, mainly that related to the effects of the ionic composition of the water on the development and productive response of the organisms (Saoud *et al.*, 2003; Roy *et al.*, 2010; Perez-Velazquez *et al.*, 2012; Valenzuela-Madrigal *et al.*, 2017). The survival around 79% obtained in the present study was similar to the reported by other authors, such as Chen *et al.* (2015) and Gao *et al.* (2016) when farmed *P. vannamei* at salinities from 2 to 3. The low survivals around 50-60% recorded in some units (SM_L and SM_H) are probably associated with problems of water quality, nutrition, or a combination of both. A low supply of dietary protein could be conduct to poor nutrition and low resistance of the organisms to stressing factors as a drastic decrease in salinity. On the other hand, an excess of protein supply is responsible for high nitrogen metabolites such as ammonia (mainly as NH_3), which provoke stress, low feed consumption decrease in oxygen transport by hemolymph, low growth, and eventually, mortalities.

An adequate ionic balance in the water column, mainly of Ca^{+2}, Mg^{+2}, and K^+, favored the physiologic response of the shrimp and improves growth, molt process, efficiency on the feed utilization, nutrients assimilation, hemolymph coagulation, nervous transmission, muscular contraction, osmoregulation, and as co-factor in diverse enzymatic processes (Davis *et al*., 2004; Cheng *et al*., 2006; Zhu *et al*., 2006). Páez-Osuna & Valencia-Castañeda (2013), suggest that the concentration of the major ions in the cultivation water must be equivalent to that contained in the seawater, which can be calculated using the equation:

$$CEx = (S)\,(R\times)$$

where CEx is the equivalent concentration of seawater, S is the salinity of the pond water, and Rx is the ratio of the seawater concentration to the normal seawater salinity.

The Rx values to estimate the acceptable concentrations of each ion in shrimp culture in low salinity water are 11.3 for potassium (K^+), 306.6 for sodium (Na^+), 11.7 for calcium (Ca^{+2}), 36.5 for magnesium (Mg^{+2}), 77.1 for sulfates (SO_4^{-2}) and 550.3 for chlorides (Cl^-). In the present study, the K^+ and Ca^{+2} values were over the recommended levels, while Mg^{+2}, Na^+, and Cl^- were under those levels (Millero, 2006).

The low concentration of Cl^- and Na^+, which should have been ≥1650.9 and 919.8 mg L^{-1} respectively, to have them in balance, probably affected the hyper-osmoregulation, since these ions are the main responsible of this metabolic process, particularly in shrimp farmed at low salinity (Davis *et al*., 2004; Huong *et al*., 2010). Otherwise, the Na^+/K^+ rate is cru-

Table 5. Productive parameters of juvenile white shrimp *Penaeus vannamei* (initial weight 1.41 ± 0.30 g) to the experimental treatments after seven weeks feeding trial. Lowercase letters in the same column indicate homogeneous subsets as determined by Tukey's test (α = 0.05). FW: final weight, WG: weight gain (%), SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio, S: survival. SM: soy meal, FM: fish meal. SM_L: diet with 25% protein; SM_M: diet with 35% protein; SM_H: diet with 45% protein (based on mixture of vegetal proteins); FM_L: diet with 22% protein; FM_M: diet with 32% protein; FM_H: diet with 41% protein (based on mixture of vegetal and animal protein); DC: diet control (commercial feed: 35% crude proteins; 7% lipids). A: source protein, B: level protein, AB: source protein and level protein.

Source protein	Level protein	FW	WG	SGR	FCR	PER	S
Vegetal	Low (SM_L)	4.4^{a}	219.4^{a}	1.0^{a}	1.7^{a}	1.7^{ab}	56.6^{ab}
	Medium (SM_M)	5.4^{abc}	288.3^{abc}	1.2^{ab}	1.6^{a}	1.4^{ab}	93.3^{c}
	High (SM_H)	4.9^{ab}	248.3^{ab}	1.1^{ab}	2.0^{a}	0.9^{a}	50.0^{a}
Animal	Low (FM_L)	6.2^{bc}	336.7^{bc}	1.3^{ab}	1.8^{a}	2.3^{ab}	90.0^{bc}
	Medium (FM_M)	6.6^{c}	389.8^{c}	1.4^{b}	1.8^{a}	2.8^{b}	90.0^{bc}
	High (FM_H)	6.2^{bc}	340.5^{bc}	1.3^{ab}	2.2^{a}	1.9^{ab}	73.3^{abc}
Control	Medium (DC)	4.9^{ab}	237.8^{ab}	1.1^{ab}	1.6^{a}	1.6^{ab}	90.0^{bc}
Effects				*P*-value			
A		0.00	0.00	0.00	0.38	0.01	0.68
B		0.20	0.28	0.26	0.08	0.22	0.39
AB		0.75	0.94	0.87	0.83	0.57	0.08

Table 6. Optimal dietary protein level for the different protein sources; polynomial equations obtained from MATLAB curve fitting toolbox. *Equation; vegetal: relationship between body weight gain (WG) and vegetal dietary protein levels; animal: relationship between body weight gain (WG) and animal dietary protein levels.

Source protein	Level protein	Weight gain (%)	*Equation	Coefficient of determination (R^2)	Optimal dietary protein level (%)
Vegetal	Low	219.4 ± 78.1	$Y = -1.3661x^2+95.112x-1400.9$	1.0	34.8
	Medium	288.3 ± 31.7			
	High	248.3 ± 88.9			
Animal	Low	336.7 ± 44.6	$Y = -1.0206x^2+59.814x-547.25$	1.0	29.3
	Medium	389.8 ± 20.6			
	High	340.5 ± 86.5			

cial for the osmoregulation process. Rates ranged from 21:1 to 23:1 during the trial, similar to what Hongyu *et al*. (2014) reported. They investigated the effect of different Na^+/K^+ rates in *Penaeus vannamei* cultures at 4, obtaining the best results at a rate of 23:1. They suggest that this rate is important for a good activity of Na^+/K^+-ATPase, responsible for the ionic interchange in the osmoregulation process.

A good protein source for animal nutrition purposes is that with a balanced content of amino acids. The recommended percentages for *P. vannamei* culture are: arginine (5.8%), methionine (2.4%), cysteine (3.6%), threonine (3.6%), valine (4.0%), isoleucine (3.4%), leucine (5.4%), lysine (5.3%), histidine (2.1%), phenylalanine (4.0%), phenylalanine + tyrosine (7.1%) and tryptophan (0.8%) (Puello-Cruz, 2013). Some amino acids are essential for diverse shrimp metabolic processes. These include the maintenance of osmolality, osmoregulation, and influence growth and survival (Perez-Velazquez *et al*., 2009). Table 3 shows the amino acid profile of experimental diets evaluates in the present study. Lysine, threonine, and alanine recorded low concentration, particularly in the diet SM_L, in which low weight gain and survival of shrimp was observed. Threonine is considered important for shrimp immune systems and influence their growth and feed assimilation efficiency (Zhou *et al*., 2013). In the present study, the content of threonine in the experimental diets ranged from 9.06 to 20.85%, within the levels required by the species which at low salinity is around 3.78% (Huai *et al*., 2009; Zhou *et al*., 2013). The levels of methionine were also low in the diets, mainly in SM_H (the diet with the higher FM replacement). The values were similar to the reported by Bauer *et al*. (2012), who found that methionine and threonine decreased as the replacement of FM increased. Methionine is considered an essential amino acid for shrimp, recommended at levels from 1.9 to 2.9 to cover the requirements of *P. vannamei* (Lin *et al*., 2015; Façanha *et al*., 2016). In this trial, the deficiency in methionine coincided with a low survival of shrimp.

The results of the present study demonstrated that both source and level of protein had a significant influence on the production parameters of shrimp farmed at low salinity (Table 5). Diets containing more FM produced the best growth, survival, and protein efficiency, which can be attributed to the desirable characteristics for the feed shrimp above, which are high digestibility and attractiveness, as well as a balanced amino acid profile (Huang *et al*., 2018).

The optimal protein level for diets based on animal sources was 29.3%, while for those based on vegetal sources, it was 34.8% (Table 6). The difference could be attributed to the amino acid profile, digestibility, and in general, the nutritional composition (Jannathulla *et al*., 2017; Huang *et al*., 2018). Additionally, diets based on vegetal sources typically contain anti-nutritional components such as lectin and folic acid (Sá *et al*., 2013), which are trypsin inhibitors (Zhou & Davis, 2015), or saponins (Xie *et al*., 2016). Which form mineral complexes affecting the nutrient absorption by shrimp (Xie *et al*., 2016; Kokou & Fountoulaki, 2018). The optimal levels of protein recommended for white shrimp farming at low salinity ranged from 30 to 36% (Kureshy & Allen-Davis, 2002; Hu *et al*., 2008; Xu & Pan, 2014). Some other authors (Liu *et al*., 2012; Gao *et al*., 2016; Huang *et al*., 2017) reported optimal values from 26 to 40%. The levels obtained in the present study are within the range indicated by the authors mentioned above. Some other factors influencing the optimal protein level for shrimp include size, environmental conditions, culture system, natural productivity, non-protein energy, and alternative ingredients (Martínez-Córdova *et al*., 2013; Shahkar *et al*., 2014). The PER of the experimental diets evaluated in this study shows that the values decreased as the protein level increased, agreeing with that reported in other studies, and it is attributed to the use of protein excess as energy source instead of mass formation (Shahkar *et al*., 2014). An important issue to consider is the carbohydrate content expressed as nitrogen free extract in the diets (NFE) (Table 3). It is interesting to observe the inverse correlation between protein level and NFE content, independently of the protein source. The NFE levels varied from 42.1 to 61.1% in diets SM, and from 41.4 to 60.9% in FM. It is well known that when *P. vannamei* is farmed at low salinity, the energetic cost for metabolic processes as osmoregulation, increases significantly (Pillai & Diwan, 2002).

This requirement could be cover by carbohydrates instead protein, permitting it to be used for tissue formation (Perez-Velazquez *et al*., 2009). Wang *et al*. (2004) reported a higher SGR for *P. vannamei* when carbohydrate supply was around 16%, in our experimental diets carbohydrate content was in the levels required by the species, and favored the idea that NFE values could be in part responsible for the results of the higher protein efficiency ratio when NFE/Protein ratio increase. However, it is important to consider this energetic source in further assays of sources and levels of protein in shrimp, mainly when a source of protein or carbohydrate would be substituted in order to avoid a decompensation in dietary energy.

It is plausible to conclude that for *P. vannamei* low salinity cultures, it is necessary an adequate ionic water balance. Where ions that are deficient (according to the shrimp requirement) must be added by using some products already recommended for aquaculture, as potassium sulfate (K_2SO_4), calcium chloride ($CaCl_2$), magnesium sulfate and potassium ($MgSO_4$-K_2SO_4), among others. It is possible to use diets based on vegetal or animal sources. Still, the level of protein must be different: 34.8 and 32.1%, respectively, because fishmeal is a better source of minerals, trace elements, amino acid profile, digestibility, attractiveness, among other characteristics desired in shrimp feed.

ACKNOWLEDGMENTS

J.C. Gil-Nuñez is grateful for financial support of Consejo Nacional de Ciencia y Tecnología with PhD scholarship. This study was supported by Instituto Tecnológico de Sonora Project PROFAPI_2020_0093 and PROFAPI_2020_0119. The authors also thank the Consejo Nacional de Ciencia y Tecnologıa for supporting project No. 1037 of Cátedras CONACYT.

REFERENCES

Ayisi, C.L., Hua, X., Apraku, A., Afriyie, G. & Kyei, B.A. 2017. Recent studies toward the development of practical diets for shrimp and their nutritional requirements. HAYATI Journal of Biosciences, 24(3): 109-117. doi: 10.1016/j.hjb.2017.09.004

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, W. & 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(1): 112-116. doi: 10.1016/j.aquaculture.2012.02.023

Bórquez-López, R.A., Casillas-Hernández, R., López-Elías, J.A., Barraza-Guardado, R.H. & Martinez-Cordova, L.R. 2018. Improving feeding strategies for shrimp farming using fuzzy logic based on water quality parameters. Aquacultural Engineering, 81: 38-45. doi: 10.1016/j.aquaeng.2018.01.002

Cabanillas-Beltrán, H., Ponce-Palafox, J.T., Arredondo-Figueroa, J.L., Esparza-Leal, H. & García-Ulloa, M. 2013. Digestibility of different thermal processed grain of legumes, *Rynchosia minima* and *Cajanus cajan*, in white shrimp *(Litopenaeus vannamei*). Asian Journal of Animal Sciences, 7(2): 36-46. doi: 10.3923/ajas.2013.36.46

Calderón, C. 2007. Metodología para determinar la variación de los componentes de nitrógeno y fosforo residual en relación con el nivel de proteína en alimentos suministrados a diferentes tallas juveniles de *Litopenaeus vannamei* (Boone). Centro de Investigaciones Biológicas del Noroeste, S.C., La Paz.

Carvalho, R.A.P.L.F., Ota, R.H., Kadry, V.O., Tacon, A.G.J. & Lemos, D. 2016. Apparent digestibility of protein, energy and amino acids of six protein sources included at three levels in diets for juvenile white shrimp *Litopenaeus vannamei* reared in high-performance conditions. Aquaculture, 465: 223-234. doi: 10.1016/j.aquaculture.2016.09.010

Chen, K., Li, E., Xu, C., Wang, X., Lin, H., Qin, J.G. & Chen, L. 2015. Evaluation of different lipid sources in diet of Pacific white shrimp *Litopenaeus vannamei* at low salinity. Aquaculture Reports, 2: 163-168. doi: 10.1016/j.aqrep.2015.10.003

Cheng, K.M., Hu, C.Q., Liu, Y.N., Zheng, S.X. & Qi, X.J. 2006. Effects of dietary calcium, phosphorus, and calcium/phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water. Aquaculture, 251(2-4): 472-483. doi: 10.1016/j.aquaculture.2005.06.022

Cummins, V.C., Rawles, S.D., Thompson, K.R., Velasquez, A., Kobayashi, Y., Hager, J. & Webster, C.D. 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture, 473: 337-344. doi: 10.1016/j.aquaculture.2017.02.022

Davis, D., Samocha, T.M. & Boyd, C.E. 2004. Acclimating Pacific white shrimp, *Litopenaeus vannamei*, to inland, low-salinity waters. Southern Regional Aquaculture Center, 2601(260): 8 pp.

Façanha, F.N., Oliveira-Neto, A.R., Figueiredo-Silva, C. & Nunes, A.J.P. 2016. Effect of shrimp stocking density and graded levels of dietary methionine over the growth performance of *Litopenaeus vannamei* reared in a green-water system. Aquaculture, 463: 16-21. doi: 10.1016/j.aquaculture.2016.05.024

Fierro, J., Rodríguez, G., León, J., Alarcón, S., Mariscal, M., Díaz, T. & Páez, F. 2018. Production and management of shrimp (*Penaeus vannamei*) in co-culture with basil (*Ocimum basilicum*) using two sources of low salinity water. Latin American Journal of Aquatic Research, 46(1): 63-71. doi: 10.3856/vol46-issue1-fulltext-8

Gao, W., Tian, L., Huang, T., Yao, M., Hu, W. & Xu, Q. 2016. Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. Aquaculture Reports, 4: 125-129. doi: 10.1016/j.aqrep.2016.09.001

González-Félixa, M.L., Perez-Velazquez, M., Ibarra-Garciaparra, G.E. & Trujillo-Villalba, J. 2017. Culture of marine sciaenids in low salinity: an opportunity for expanded aquaculture in Mexico. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D.A., Gamboa-Delgado, J., López Acuña, L.M. & Galaviz-Espinoza, M. (Eds.). Investigación y desarrollo en nutrición acuícola. Universidad Autónoma de Nuevo León, Nuevo León, pp. 156-170.

Hongyu, L., Tan, B., Yang, J., Lin, Y., Chi, S., Dong, X. & Qihui, Y. 2014. Effect of various Na/K ratios in low-salinity well water on growth performance and physiological response of Pacific white shrimp *Litopenaeus vannamei*. Chinese Journal of Oceanology and Limnology, 32(5): 991-999.

Hu, Y., Tan, B., Mai, K., Ai, Q., Zheng, S. & Cheng, K. 2008. Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. Aquaculture Nutrition, 14(6): 499-506. doi: 10.1111/j.1365-2095.2007.00555.x

Huai, M.Y., Tian, L.X., Liu, Y.J., Xu, A.L., Liang, G.Y. & Yang, H.J. 2009. Quantitative dietary threonine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone) reared in low-salinity water. Aquaculture Research, 40(8): 904-914. doi: 10.1111/j.1365-2109.2009.02181.x

Huang, F., Wang, L., Zhang, C. & Song, K. 2017. Replacement of fishmeal with soybean meal and mineral supplements in diets of *Litopenaeus vannamei* reared in low-salinity water. Aquaculture, 473: 172-180. doi: 10.1016/j.aquaculture.2017.02.011

Huang, Y.J., Zhang, N.N., Fan, W.J., Cui, Y.Y., Limbu, S.M., *et al*. 2018. Soybean and cottonseed meals are good candidates for fishmeal replacement in the diet of juvenile *Macrobrachium nipponense*. Aquaculture International, 26(1): 309-324. doi: 10.1007/s10499-017-0215-1

Huong, D.T.T., Jasmani, S., Jayasankar, V. & Wilder, M. 2010. Na/K-ATPase activity and osmo-ionic regulation in adult whiteleg shrimp *Litopenaeus vannamei* exposed to low salinities. Aquaculture, 304(1-4): 88-94. doi: 10.1016/j.aquaculture.2010.03. 025

Jannathulla, R., Dayal, J.S., Ambasankar, K., Khan, H.I., Madhubabu, E.P. & Muralidhar, M. 2017. Effect of protein solubility of soybean meal on growth, digestibility and nutrient utilization in *Penaeus vannamei*. Aquaculture International, 25(5): 1693-1706. doi: 10.1007/s10499-017-0147-9

Jarwar, A.A.M. 2015. Water quality in inland saline aquaculture ponds and its relationships to shrimp survival and production. PhD Thesis. Auburn University, Auburn.

Jatobá, A., Vieira, F., da Silva, B.C., Soares, M., Mouriño, J.L.P. & Seiffert, W.Q. 2017. Replacement of fishmeal for soy protein concentrate in diets for juvenile *Litopenaeus vannamei* in biofloc-based rearing system. Revista Brasileira de Zootecnia, 46(9): 705-713. doi: 10.1590/S1806-92902017000900001

Katya, K., Lee, S., Yun, H., Dagoberto, S., Browdy, C.L., Vazquez-Anon, M. & Bai, S.C. 2016. Efficacy of inorganic and chelated trace minerals (Cu, Zn and Mn) premix sources in Pacific white shrimp, *Litopenaeus vannamei* (Boone) fed plant protein based diets. Aquaculture, 459(1): 117-123. doi: 10.1016/j.aquaculture.2016.03.033

Kokou, F. & Fountoulaki, E. 2018. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture, 495(1): 295-310. doi: 10.1016/j.aquaculture.2018. 06.003

Kureshy, N. & Allen-Davis, D. 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 204(1-2): 125-143. doi: 10.1016/S0044-8486(01)00649-4

Li, H., Lan, R., Peng, N., Sun, J. & Zhu, Y. 2016. High resolution melting curve analysis with MATLAB-based program. Measurement, 90: 178-186. doi: 10.1016/j.measurement.2016.04.057

Lim, C. 1996. Substitution of cottonseed meal for marine animal protein in diets for *Penaeus vannamei*. Journal of the World Aquaculture Society, 27(4): 402-409.

Lim, C. 1997. Replacement of marine animal protein with peanut meal in diets for juvenile white shrimp, *Penaeus vannamei*. Journal of Applied Aquaculture, 7(3): 67-78. doi: 10.1300/J028v07n03

Lin, H., Chen, Y., Niu, J., Zhou, C., Huang, Z., Du, Q. & Verdana, J.Z. 2015. Dietary methionine requirements of Pacific white shrimp *Litopenaeus vannamei*, of three different sizes. Israeli Journal of Aquaculture - Bamidgeh, 67: 10 pp.

Liu, X., Ye, J., Wang, K., Kong, J., Yang, W. & Zhou, L. 2012. Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Research, 43(5): 745-755. doi: 10.1111/j.1365-2109.2011.02883.x

Luo, L., Wang, J., Pan, Q., Xue, M., Wang, Y., Wu, X. & Li, P. 2012. Apparent digestibility coefficient of poultry by-product meal (PBM) in diets of *Penaeus monodon* (Fabricius) and *Litopenaeus vannamei* (Boone), and replacement of fishmeal with PBM in diets of *P. monodon*. Aquaculture Research, 43(8): 1223-1231. doi: 10.1111/j.1365-2109.2011.02926.x

Martínez-Córdova, L.R., Porchas-Martínez, M., Miranda, A., Baeza, E. & López, J.A. 2013. Selección de alimentos y estrategias de alimentación acordes a las condiciones de cultivo del camarón. In: Cruz-Suarez, L.E., Rique-Marie, D., Tapia-Salazar, M., Nieto-Lopez, M., Villarreal-Cavazos, A., Gamboa-Delgado, J. & Alvarez-Gonzalez, C. (Eds). Contribuciones recientes en alimentación y nutrición acuícola. Universidad Autónoma de Nuevo León, Nuevo León, pp. 84-104.

MathWorks. 2012. MATLAB Release 2012b. The MathWorks, Inc., Natick.

Millero, F. 2006. Chemical oceanography. CRC Press, Boca Raton.

Moreno-Arias, A., López-Elías, J.A., Martínez-Córdova, L.R., Ramírez-Suárez, J.C., Carvallo-Ruiz, M.G., García-Sánchez, G. & Miranda-Baeza, A. 2018. Effect of fishmeal replacement with a vegetable protein mixture on the amino acid and fatty acid profiles of diets, biofloc and shrimp cultured in BFT system. Aquaculture, 483: 53-62. doi: 10.1016/j.aquaculture. 2017.10.011

National Research Council (NRC). 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Columbia.

Páez-Osuna, F. & Valencia-Castañeda, G. 2013. Calidad de agua en cultivo de camarón blanco (*Litopenaeus vannamei*) con baja salinidad. In: Covarrubias, M.S.M. (Ed.). Camaronicultura en agua de baja salinidad. Editorial Trillas, Ciudad de México.

Panini, R.L., Freitas, L.E.L., Guimarães, A.M., Rios, C., Da Silva, M.F.O., Vieira, F.N., *et al*. 2017. Potential use of mealworms as an alternative protein source for Pacific white shrimp: digestibility and performance. Aquaculture, 473: 115-120. doi: 10.1016/j.aquaculture.2017.02.008

Panini, R.L., Pinto, S.S., Nóbrega, R.O., Vieira, F.N., Fracalossi, D.M., Samuels, R.I., *et al*. 2017. Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp *Litopenaeus vannamei*. Food Research International, 102: 445-450. doi: 10.1016/J.FOODRES.2017.09.017

Peña-Rodríguez, A., Elizondo-González, R., Nieto-López, M.G., Ricque-Marie, D. & Cruz-Suárez, L.E. 2017. Practical diets for the sustainable production of brown shrimp, *Farfantepenaeus californiensis*, juveniles in presence of the green macroalga *Ulva clathrata* as natural food. Journal of Applied Phycology, 29(1): 413-421. doi: 10.1007/s10811-016-0846-z

Perez-Velazquez, M., Davis, D.A., Roy, L.A. & González-Félix, M.L. 2012. Effects of water temperature and Na+:K + ratio on physiological and production parameters of *Litopenaeus vannamei* reared in low salinity water. Aquaculture, 342-343(1): 13-17. doi: 10.1016/j.aquaculture.2012.02.008

Pérez-Velázquez, M., Martínez-Porchas, M., González-Félix, M.L. & Martínez-Córdova, L.R. 2009. El cultivo de camarón blanco del Pacífico *Litopenaeus vannamei* (Boone) en baja salinidad, como una ópcion de acuicultura sustentable. In: Martínez-Córdova, L.R. (Ed.). Camaronicultura sustentable: manejo y evaluación. Editorial Trillas, Ciudad de México.

Pillai, B.R. & Diwan, A.D. 2002. Effects of acute salinity stress on oxygen consumption and ammonia excretion rates of the marine shrimp *Metapenaeus monoceros*. Journal of Crustacean Biology, 22(1): 45-52.

Puello-Cruz, A.C. 2013. Nutrición en cultivo de camarón blanco (*Litopenaeus vannamei*) con baja salinidad. In: Morales-Covarrubias, M.S. (Ed.). Camaronicultura en agua de baja salinidad. Editorial Trillas, Ciudad de México, pp. 24-40.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. & Boyd, C.E. 2010. Shrimp culture in inland low salinity waters. Reviews in Aquaculture, 2(4): 191-208. doi: 10.1111/ j.1753-5131.2010.01036.x

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*. Aquaculture Nutrition, 19(2): 199-210. doi: 10.1111/j.1365-2095.2012.00954.x

Saoud, I.P., Davis, D.A. & Rouse, D.B. 2003. Suitability studies of inland well waters for *Litopenaeus vannamei* culture. Aquaculture, 217(1-4): 373-383. doi: 10.1016/S0044-8486(02)00418-0

Shahkar, E., Yun, H., Park, G., Jang, I.K., Kyoung Kim, S., Katya, K. & Bai, S.C. 2014. Evaluation of optimum dietary protein level for juvenile whiteleg shrimp (*Litopenaeus vannamei*). Journal of Crustacean Biology, 34(5): 552-558. doi: 10.1163/1937240X-00002267

Valenzuela-Madrigal, I.E., Valenzuela-Quiñónez, W., Esparza-Leal, H.M., Rodríguez-Quiroz, G. & Aragón-Noriega, E.A. 2017. Effects of ionic compo-sition on growth and survival of white shrimp *Litopenaeus vannamei* culture at low-salinity well water. Revista de Biología Marina y Oceanografía, 52(1): 103-112. doi: 10.4067/S0718-19572017000 100008

Van Wyk, P. 1999. Principles of recirculating system design. In: Production of marine shrimp in freshwater recirculating aquaculture systems. Florida Department of Agriculture and Consumer Services, Florida.

Vázquez-Ortiz, F.A., Caire, G., Higuera-Ciapara, I. & Hernández, G. 1995. High performance liquid chromatographic determination of free amino acids in shrimp. Journal of Liquid Chromatography, 18(19): 2059-2068.

Wang, X., Ma, S., Dong, S. & Cao, M. 2004. Effects of salinity and dietary carbohydrate levels on growth and energy budget of juvenile *Litopenaeus vannamei*. Journal of Shellfish Research, 23: 231-236.

Xie, S., Liu, Y., Zeng, S., Niu, J. & Tian, L. 2016. Partial replacement of fishmeal by soy protein concentrate and soybean meal based protein blend for juvenile Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 464: 296-302. doi: 10.1016/j.aquaculture.2016.07.002

Xu, W.J. & Pan, L.Q. 2014. Evaluation of dietary protein level on selected parameters of immune and antioxidant systems and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. Aquaculture, 426-427: 181-188. doi: 10.1016/j.aquaculture.2014.02.003

Zhou, Y. & Davis, A. 2015. Improved high soy shrimp feeds for the pacific white shrimp *Litopenaeus vannamei*. In: Suárez, E.L.C., Marie, R., Salazar, M.T., López, M.G.N., Villarreal, D.A. & Delgado, J.G. (Eds.). Nutrición acuícola: investigación y desarrollo, Universidad Autónoma de Nuevo León, Nuevo León, pp. 1-22.

Zhou, Q.C., Wang, Y.L., Wang, H.L. & Tan, B.P. 2013. Dietary threonine requirements of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 392-395: 142-147. doi: 10.1016/j.aquaculture.2013.01.026

Zhu, C.B., Dong, S.L., Wang, F. & Zhang, H.H. 2006. Effects of seawater potassium concentration on the dietary potassium requirement of *Litopenaeus vannamei*. Aquaculture, 258(1-4): 543-550. doi: 10.1016/j.aquaculture.2006.03.038

Received: 6 June 2019; Accepted: 31 January 2020