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PROTEIN REQUIREMENT FOR INITIAL REARING PHASE OF PACIFIC WHITE SHRIMP IN BIOFLOC SYSTEM*

Fernanda Morais HENRIQUES[1]
Ariane Martins GUIMARÃES[1]
Carlos Andrés DÍAZ[1]
Débora Machado FRACALOSSI[2]
Edemar Roberto ANDREATTA[1]
Felipe do Nascimento VIEIRA[1]*

[1]Universidade Federal de Santa Catarina – UFSC, Departamento de Aquicultura, Laboratório de Camarões Marinhos – LCM. Beco dos Coroas, Barra da Lagoa, 88.061600, Florianópolis, SC, Brazil. felipe.vieira@ufsc.br (*corresponding author).

[2]Universidade Federal de Santa Catarina – UFSC, Departamento de Aquicultura, Laboratório de Nutrição de Organismos Aquáticos – Labnutri. Rod. Francisco Thomaz dos Santos, Armação, 88.066-260, Florianópolis, SC, Brazil.

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ABSTRACT

This study evaluated the protein requirement of *Litopenaeus vannamei* post-larvae during the initial rearing phase in a biofloc system. Five different diets were evaluated with increasing concentrations of crude protein: 31.28, 36.29, 41.57, 46.34, and 51.74 g 100 g^{-1} CP. Post-larvae (0.16 ± 0.01 g) were stocked at a density of 450 PL m^{-3} in 400 L tanks. Water quality parameters were maintained within the limits recommended for shrimp farming. After 38 days, a regression analysis revealed that levels of CP content (65.29–72.83%), EE (10.45–11.65%) and body N (10.45–11.64%) increased with increasing protein levels in the diet. A similar trend was observed in the biofloc sludge with respect to CP and N. Survival exceeded 80%, and the shrimp with diets containing 31.28 to 46.34 g 100 g^{-1} CP presented an increase in final weight (1.52–2.61 g), productivity (0.69–1.10 kg m^{-3}), weight gain (1.38–2.44 g), and feeding efficiency (77.28–101.68%), whereas these indices decreased to 51.74 g 100 g^{-1} CP. Crude protein content from 44.26 to 47.12 g 100 g^{-1} provided the best growth performance during the initial rearing phase of Pacific white shrimp *L. vannamei* in a biofloc system.

Keywords: *Litopenaeus vannamei*; nutrition; diets; BFT.

EXIGÊNCIA PROTEICA PARA A FASE INICIAL DO CAMARÃO-BRANCO-DO-PACÍFICO EM SISTEMA DE BIOFLOCOS

RESUMO

Esse estudo avaliou a exigência proteica do *Litopenaeus vannamei* na fase inicial de cultivo em sistema de bioflocos. Cinco dietas com quantidades crescentes de proteína bruta (31,28; 36,29; 41,57; 46,34 e 51,74 g 100 g^{-1} PB) foram avaliadas. As pós-larvas (0,16 ± 0,01 g) foram estocadas na densidade de 450 PL m^{-3} em tanques de 400 L. A qualidade de água manteve-se dentro dos limites adequados para o cultivo. Após 38 dias, uma análise de regressão revelou que teores de PB (65,29–72,83%), EE (10,45–11,65%) e N corporal (10,45–11,64%) aumentaram com os níveis crescente de proteína na dieta. A mesma análise foi realizada para o lodo do bioflocos, que apresentou aumento crescente de PB e N. A sobrevivência foi superior a 80% e os camarões alimentados com dietas contendo 31,28 à 46,34g 100 g^{-1} PB obtiveram aumento no peso final (1,52–2,61 g), produtividade (0,69–1,10 kg m^{-3}), ganho em peso (1,38–2,44 g) e eficiência alimentar (77,28–101,68%), enquanto esses índices decresceram no tratamento 51,74 g 100 g^{-1} PB. O conteúdo de proteína bruta entre 44,26 à 47,12 g 100 g^{-1} PB proporocionou o melhor desempenho de crescimento durante a fase inicial do cultivo do camarão-branco-do-pacífico *L. vannamei* em bioflocos.

Palavras-chave: *Litopenaeus vannamei*; nutrição; dietas; BFT.

INTRODUCTION

Marine shrimp are among the most valued and reared crustaceans in the world. In 2018, the production of *Litopenaeus vannamei* (Boone, 1931), the main representative of the group, reached 4,966,2 million tonnes, about 53% of the world's total produced crustaceans (FAO, 2020). However, disease and environmental damage pose serious threats to the aquaculture sector, and some management strategies have been adopted to solve them, allowing the production of these peneids to continue growing. Some of the main practices involve proper management, water quality control and the provision of quality diets to animals (Shiau, 1998; Soares et al., 2015; Xu et al., 2016).

In this context, the biofloc system (BFT) is an alternative rearing mechanism that contributes to shrimp farming because it maintains water quality without exchanging the water, and it provides shrimp with complementary feed resources. Specifically, microbial flocs are small particles composed of bacteria, algae, fungi, and organic and inorganic fragments that are available in rearing water. These particles offer such nutrients as lipids, carbohydrates, vitamins, and proteins (Crab et al., 2010; Jatobá et al., 2014; Xu et al., 2016), and contribute to a decrease in the apparent feed conversion ratio (Krummenauer et al., 2020).

In general, *Litopenaeus vannamei* requires 20 a 50% crude protein (CP) in its diet, depending on its stage of life (Smith et al., 1985; Kanazawa, 1989; Jatobá et al., 2014; Xu and Pan, 2014; Pragnelli et al., 2016). The protein requirement is higher in the early stages of shrimp life (Smith et al., 1985; Shearer, 2002).

Nutrition studies performed on the BFT system show that 29% of total food intake by *L. vannamei* juveniles is possibly derived from microbial aggregates. Some point out that it is possible to reduce feed protein levels from 35% to 25% without compromising juvenile growth performance indices (Burford et al., 2003; Wasielesky et al., 2006; Crab et al., 2010; Melo et al., 2015).

Therefore, the availability of bioflocs as a source of nutrients may contribute to a reduction in feed provision, as well as the demand for protein in the diet (Xu et al., 2012). This approach can result in a considerable reduction in overhead (Krummenauer et al., 2020), as feed can represent about 50% of production costs, while minimizing the amount of nitrogen introduced into water from diet enclosures (McIntosh et al., 2001).

Therefore, the objective of this work was to evaluate the dietary protein requirements of the Pacific white shrimp during the initial phase of rearing in a biofloc system and the effect of such supplementation on water quality and growth performance indices.

MATERIALS AND METHODS

The study was conducted over the course of 38 days in the Marine Shrimp Laboratory (LCM) of the Universidade Federal de Santa Catarina (UFSC) in Florianópolis, SC, Brazil. Post-larvae at the 5-day stage (PL5) of Speed Line lineage (high growth performance *L. vannamei)* were obtained from Aquatec Ltda, Rio Grande do Norte, Brazil. Post-larvae were reared in a biofloc system until reaching a weight of 0.16 g (~PL51) and then transferred to the experimental units.

The experiment focused on the initial rearing phase of *L. vannamei*. During this time, the shrimp received five different diets with increasing concentrations of crude protein (CP): 31.28; 36.29; 41.57; 46.34 and 51.74 g 100 g^{-1}. The formulation of the diets and the analysis of these rations were carried out by the Aquatic Organism Nutrition Laboratory (Labnutri) at the UFSC Department of Aquaculture.

The diets were formulated using Optimal Formula 2000 software and were based on the NRC (2011) recommendations and dietary requirements for excellent performance of *L. vannamei* post-larvae. For information not found, *Penaeus monodon* was used as a reference (NRC, 2011) based on the mathematical conversion according to its post-larvae requirements for crude protein (Millamena et al., 1996a, 1996b, 1997, 1998, 1999). The main sources of protein were analyzed at CBO Ltd. (Supplier of Bromatological Products and Technical Analyses): salmon by product meal (72.32 g 100 g^{-1} crude protein, 11.76 g 100 g^{-1} crude fat, 0.07 g 100 g^{-1} crude fiber, 15.25 g 100 g^{-1} minerals, 88.11 g 100 g^{-1} dry matter and 4,990.32 kcal kg^{-1} crude energy) and soybean meal (51.47 g 100 g^{-1} crude protein, 3.34 g 100 g^{-1} crude fat, 3.71 g 100 g^{-1} crude fiber, 6.45 g 100 g^{-1} minerals, 89.65 g 100 g^{-1} dry matter and 4,350.25 kcal kg^{-1} crude energy).

The ingredients were balanced to ensure the iso-energetic and iso-lipidic content of the different diets (Table 1). Salmon by-product meal (72.32% CP) and soybean meal (51.46% CP), were incorporated at a ratio of 1.7 (salmon: soybean), as the source of protein.

Each diet was processed separately, pelletized (1.5 mm matrix), and the pellets dried in a forced air circulation oven at 50°C for approximately 1.5 hours. Once ready, the expanded feed was ground in a mortar to 1.5 mm and then frozen until use to minimize oxidation and the loss of fatty acids. The amino acid profile of the experimental diets was evaluated to ensure the absence of deficiencies based on the nutritional recommendations adopted for the present study (Table 2).

The experimental units were assigned randomly, with three replicates of each treatment in 15 circular polyethylene tanks containing 400 L of water. These tanks were kept in an isolated room under artificial light (12 hours light, 12 hours dark) and equipped with 800 W water heaters controlled by thermostats (28-29ºC). The AeroTube aerationsystem maintained dissolved oxygen at over 5 mg L^{-1} and suspended solids in the water column. Six artificial substrates made of Needlona cloth were added to each tank to increase the available area by 100%. This facilitated the fixation of the biofilm and contributed to the dispersal of shrimp in the tanks.

The experimental units were filled with 100% bioflocs from a matrix tank. The initial water conformed to the following measured characteristics: salinity, 28.70 g L^{-1}; pH 7.96; alkalinity, 108 mg L^{-1}; ammonia, 0.00 mg L^{-1}; nitrite, 8.36 mg L^{-1}; nitrate, 31.24 mg L^{-1}, and total suspended solids (TSS), 275 mg L^{-1}. These values were consistent with bioflocs at the initial stage of maturation based on the level of nitrification, owing to the presence of nitrite and low level of nitrate (Ebeling et al., 2006).

The tanks were stocked with shrimp at a mean body weight of 0.16 ± 0.01 g. Shrimp were counted for an initial density of 450 PL m^{-3}. The water was not exchanged during the experiment, except to top off the tanks to compensate for evaporation.

Feed was distributed four times each day (08:00 h, 11:00 h, 14:00 h and 17:00 h). Daily feeding rates were 15% of shrimp body weight at the beginning of the experiment and then gradually decreased to 8%, according to Van Wyk (1999). This schedule was adjusted weekly, following biometric monitoring, which, in turn, resulted in an adjustment in feeding rates according to shrimp biomass. Feed consumption was checked by sampling the water with a hand net (700 µm mesh) passed between artificial

Table 1. Composition of the experimental diets for the development of Pacific white shrimp *Litopenaeus vannamei* post-larvae in a biofloc system containing different levels of crude protein content.

Ingredient (g 100 g^{-1})	Treatments (Crude protein g 100 g^{-1})				
	31.28	**36.29**	**41.57**	**46.34**	**51.74**
Salmon by-product meal	25.26	31.34	37.00	42.50	48.17
Soybean meal	14.90	18.00	21.50	24.89	27.80
Wheat flour	13.22	12.00	12.00	11.00	9.00
Rice grits	30.00	20.41	10.26	3.65	0.00
Cod liver oil	3.60	3.20	3.00	1.80	0.99
Vitamin premix[1]	0.38	0.38	0.38	0.38	0.38
Vitamin C	0.07	0.07	0.07	0.07	0.07
Macromineral premix	6.62	6.62	6.62	6.62	6.62
Micromineral premix[2]	1.62	1.62	1.62	1.63	1.63
Lecithin	2.05	2.05	2.05	2.05	2.05
Carboxymethylcellulose	2.00	2.00	2.00	2.00	2.00
Kaolin	0.29	2.31	3.50	3.41	1.29
Proximate composition					
Moisture (g 100 g^{-1})	11.57	11.02	10.11	11.13	11.76
Crude protein (g 100 g^{-1})	31.28	36.29	41.57	46.34	51.74
Crude fat (g 100 g^{-1})	9.56	9.98	9.62	9.92	10.04
Ash (g 100 g^{-1})	11.67	14.66	17.41	18.64	17.97
Gross energy (kcal kg^{-1}) [3]	4,100.00	4,100.00	4,143.83	4,176.52	4,301.15

[1] Levels per kg of the product: vitamin A, 900 mg; vitamin D, 25 mg; vitamin E, 46,900 mg; vitamin K, 14,000 mg; vitamin B12, 50 mg; biotin, 750 mg; folic acid, 3,000 mg; niacin, 70,000 mg; pantothenic acid, 40,000 mg; vitamin B6, 33,000 mg; riboflavin, 20,000 mg; thiamin, 30,000 mg. [2] Levels per kg of the product: copper, 23,330 mg; manganese, 6,500 mg; selenium, 125 mg; zinc, 100,000 mg, iodine, 1,000 mg; cobalt, 50 mg, magnesium, 20 mg; potassium, 6.1 mg. [3] Gross energy calculated.

Table 2. Essential amino acid profile for each dietary formula for Pacific white shrimp *Litopenaeus vannamei* post-larvae in a biofloc system containing different levels of crude protein content.

Essential amino acid (g 100 g^{-1})	Treatments (Crude protein g 100 g^{-1})					Requirement[1] (g 100 g^{-1})
	31.28	**36.29**	**41.57**	**46.34**	**51.74**	
Arginine	2.03	2.37	2.71	3.05	3.40	1.85[2]
Histidine	0.84	0.98	1.13	1.28	1.42	0.76[3]
Isoleucine	1.21	1.40	1.60	1.80	2.00	0.96[3]
Lysine	1.84	2.20	2.55	2.90	3.25	1.61[2]
Leucine	1.98	2.28	2.58	2.89	3.20	1.82[3]
Methionine	0.72	0.84	0.97	1.09	1.22	0.84[4]
Methionine+Cystein	1.19	1.39	1.58	1.78	1.98	1.23[4]
Phenylalanine	1.26	1.45	1.64	1.83	2.02	1.32[3]
Phenylalanine+Tyrosine	2.22	2.55	2.90	3.24	3.59	2.16[3]
Tyrosine	0.95	1.10	1.26	1.41	1.57	0.84[5]
Threonine	1.22	1.44	1.65	1.87	2.08	1.23[6]
Tryptophan	0.26	0.29	0.32	0.35	0.38	0.19[3]
Valine	1.48	1.73	1.98	2.23	2.47	1.28[7]

[1] Based on the mathematical conversion according to the requirements of the post-larvae of *Penaeus monodon*, in terms of crude protein: [2] Millamena et al. (1998); [3] Millamena et al. (1999); [4] Millamena et al. (1996a); [5] NRC (2011); [6] Millamena et al. (1997); [7] Millamena et al. (1996b).

substrates two hours after feeding. When leftover feed was found in two consecutive samples, the feeding rate was reduced by 10%.

To control the nitrogenous compounds, white sugar was added to the water to neutralize the ammonia excreted by the shrimp (Chamorro-Legarda et al., 2016). It was assumed that 75% of the nitrogen would be transformed into ammonia dissolved in the water, making it necessary to use 15.17 g of carbohydrate to neutralize each gram of ammonia (Ebeling et al., 2006). In addition, when the concentration of ammonia exceeded 1 mg L^{-1}, an extra dose of sugar was added to the water at the same ratio (15.17 g sugar: 1 g ammonia). To maintain alkalinity over 120 mg L^{-1} and to guarantee the buffer effect of the system, hydrated lime was added to the water whenever alkalinity concentration fell below the recommended level (<120 mg L^{-1}). The concentrations of both sugar and $CaCO_3$ in the water were measured during the experiment. No solids were removed from the water during the trial.

At the end of the experiment, the experimental diets, samples of whole shrimp (~300 g from each tank) and biofloc sludge were all sent to Labnutri at UFSC for analysis according to the approach described by the Association of Official Analytical Chemists (AOAC, 1999). The diets and shrimp were dried at 105°C for the analysis of dry matter and incinerated at 550°C to determine the ash content, protein (N were measured by Kjeldalh method and protein value obtained multiplying the N content obtained per 6.25) and ethereal extract (Soxhlet extractor, following acid hydrolysis). Biofloc sludge was also analyzed for crude protein (N were measured by Kjeldalh method and protein value obtained multiplying the N content obtained per 6.25) and nitrogen.

Shrimp were weighted obtained each week to determine the growth of the animals and adjust the amount of feed. At the end of the experiment, the following parameters were determined: Final weight (g) = final biomass/number of shrimp; Survival (%) = (initial number of shrimp/final number) x 100; Gain in biomass (g) = final biomass – initial biomass; Weight gain (g) = (final biomass – initial biomass)/number of shrimp; Feeding efficiency (%) = (weight gain/total ration [dry matter]) x 100; Productivity (kg m^{-3}) = final biomass (kg)/the volume of the tank (m^3).

Dissolved oxygen and temperature (YSI 55 oximeter), salinity (YSI 30 digital salinometer) were measured twice daily, while nitrate (kit commercial, Hach ACA01), pH (YSI 100 pHmeter) and TSS (APHA, 2005) were measured once a week. Alkalinity (APHA, 2005), total ammonia and nitrite (APHA, 2005) were measured twice a week.

Once the assumptions of normality and homoscedasticity had been tested, water quality was analyzed using a one-way ANOVA, supplemented with Tukey test (p <0.05). Growth performance indices and the composition of shrimp and biofloc sludge was analyzed using polynomial regression with the significance of the coefficients evaluated using ANOVA (p <0.05). The maximum (X_{max}) weight gain and feed efficiency were calculated using the quadratic regression and protein requirement was determined as 95% of $X_{max.}$

RESULTS

Water Quality

The water quality parameters obtained in the present experiment are shown in Table 3.

Composition of shrimp and biofloc sludge

The composition of shrimp and biofloc sludge obtained in the present experiment are shown in Table 4.

System inputs

The amount of sugar and calcium hydroxide where higher in the tanks fed with the diets with higher crude protein content (Table 5).

Table 3. Water quality parameters (mean ± standard deviation) recorded during culture of Pacific white shrimp *Litopenaeus vannamei* post-larvae in a biofloc system containing different levels of crude protein (n = 3).

Variable	Treatments (Crude protein g 100 g^{-1})				
	31.28	**36.29**	**41.57**	**46.34**	**51.74**
Dissolved Oxygen (mg L^{-1})	5.66±0.03	5.64±0.11	5.65±0.05	5.66±0.01	5.60±0.03
Temperature (ºC)	28.27±0.18	28.29±0.49	28.23±0.34	28.22±0.24	28.22±0.21
pH	7.84±0.01^{b}	7.88±0.01^{b}	7.89±0.00^{ab}	7.91±0.01^{a}	7.90±0.01^{a}
Salinity (g L^{-1})	30.30±0.09^{a}	30.12±0.16^{a}	30.21±0.20^{a}	30.27±0.21^{a}	30.56±0.07^{a}
Alkalinity (mg $CaCO_3$ L^{-1})	150.44±0.85	151.42±4.88	147.63±4.87	148.17±5.70	146.52±2.30
Ammonia (mg L^{-1})	0.09±0.02	0.14±0.05	0.12±0.02	0.15±0.01	0.10±0.02
Nitrite (mg L^{-1})	1.93±1.05	2.03±0.69	2.65±1.24	2.56±0.79	3.59±0.07
Nitrate (mg L^{-1})	15.74±3.15	15.33±3.90	19.46±2.64	18.20±3.14	21.06±0.66
Orthophosphate (mg L^{-1})	1.58±0.07	1.63±0.16	1.69±0.02	1.58±0.05	1.57±0.15
Total Suspended Solids (mg L^{-1})	246.87±21.83	265.00±12.95	248.23±4.6	275.80±26.77	266.90±5.38
Volatile Suspended Solids (%)	29.82±2.12^{b}	31.45±2.13^{ab}	30.81±3.07^{ab}	36.55±1.09^{a}	32.92±3.23^{ab}
Fixed Solids (%)	70.18±2.12^{a}	68.55±2.13^{ab}	69.19±3.07^{ab}	63.45±1.09^{b}	67.08±3.23^{ab}

Means followed by equal letters do not differ by Tukey test (p ≥0.05).

Table 4. Composition (dry weight; mean ± standard deviation) of Pacific white shrimp *Litopenaeus vannamei* post-larvae and biofloc sludge (%) after 38 days of rearing in a biofloc system containing different levels of crude protein (CP).[1]

Treatment (g 100 g^{-1} CP)	Moisture (%)	Crude protein (%)	Ethereal extract (%)	Ash (%)	Nitrogen (%)
Shrimp					
31.28	22.60±2.23	65.29±7.58	10.45±2.13	4.66±0.38	10.45±1.21
36.29	22.83±2.29	69.23±1.86	11.08±0.68	4.73±0.26	11.08±0.30
41.57	23.51±1.38	70.38±2.24	11.26±0.31	3.95±0.26	11.26±0.36
46.34	23.64±4.42	72.14±0.40	11.54±0.59	5.02±0.31	11.54±0.06
51.74	25.17±0.83	72.83±1.28	11.65±0.53	3.52±0.25	11.65±0.20
Bioflocs sludge (%)					
31.28		18.91±7.82	-	-	3.03±1.25
36.29		13.46±2.48	-	-	2.15±0.40
41.57	4.68[2]	16.06±2.03	-	-	2.57±0.32
46.34		20.81±1.29	-	-	3.33±0.21
51.74		24.31±7.66	-	-	3.89±1.22

[1] Average bioflocs sludge (%). [2] Five sludge samples were pooled for easy handling and processing.

Table 5. Amount of sugar and calcium hydroxide (mean ± standard deviation) added to the water during the initial rearing phase of Pacific white shrimp *Litopenaeus vannamei* post-larvae in a biofloc system containing different levels of crude protein (CP) (n = 3).

Treatment (g 100 g^{-1} CP)	Sugar applied (g)	Calcium hidroxide (g)
31.28	98.47±18.54	39.14±3.35
36.29	200.60±62.12	43.73±4.16
41.57	201.82±41.99	50.72±3.92
46.34	327.00±25.83	50.48±3.82
51.74	301.15±71.60	54.60±6.92

Growth performance

Weight gain and feeding efficiency increased 1.38-2.44 g and 77.28-101.68%, respectively (Figure 1), as well as final weight (1.52–2.61 g), gain in biomass (248.92–409.89 g), and productivity (0.69–1.10 kg m^{-3}), albeit with a final decrease in all these indices in the 51.74g 100 g^{-1} CP treatment (Table 6). Shrimp survival was above 80%. Protein requirement, considering weight gain and feed efficiency was calculated to be between 42.05 and 44.76 g 100 g^{-1}.

DISCUSSION

Water Quality

The water quality parameters (Table 3) were all within the limits recommended for the farming of marine shrimp (Van Wyk and Scarpa, 1999). While the pH, salinity and suspended volatile and fixed solids did vary significantly among the treatments, no adverse effect was observed in shrimp performance, given that the values were within the optimal range for the species (Mishra et al., 2008). Total suspended solids were invariably below 400 mg L^{-1}, as recommended by Schveitzer et al. (2013). Nitrate was decreased during the experiment probably by the action of the heterotrophic bacteria (Schneider et al., 2007).

The nitrification process involves the conversion of ammonia to nitrite and then nitrite to nitrate (Hargreaves, 2013). No prominent nitrification was observed at the beginning of the trials. This resulted in an accumulation of nitrite during the first week of treatment with the highest concentration of CP (51.74 g 100 g^{-1}), finally reaching ~11 mg L^{-1} N-NO_2. Nor did it increase the amount of nitrate or reduce alkalinity, which usually occurs during nitrification (Ebeling et al., 2006). Even so, the nitrite and ammonia concentrations recorded during the present study were within the recommended range for cultivation (Lin and Chen, 2001, 2003).

Correia et al. (2014) evaluated commercial foods with 30% and 40% CP and observed a continuous increase of nitrite with conversion to nitrate occurring only after one week. The authors attributed these results to the slow growth of nitrite-oxidizing bacteria (NOB) and to lower nitrogen uptake and nitrification rates by microbial biomass compared to rates when nitrogen is added to the system, a fact also noted in this paper.

McIntosh et al. (2001) concluded that the protein content of foods results in an increase in ammonia concentration in closed systems. In general, high dietary protein content results in increased nitrogen input to the ration and, hence, the increasing need for the application of sugar as a source of organic carbon to balance the nitrogenous compounds present in the water (Chamorro-Legarda et al., 2016). So, it was necessary to add white sugar during the present study to neutralize the ammonia through the action of heterotrophic bacteria (Ebeling et al., 2006). This finding

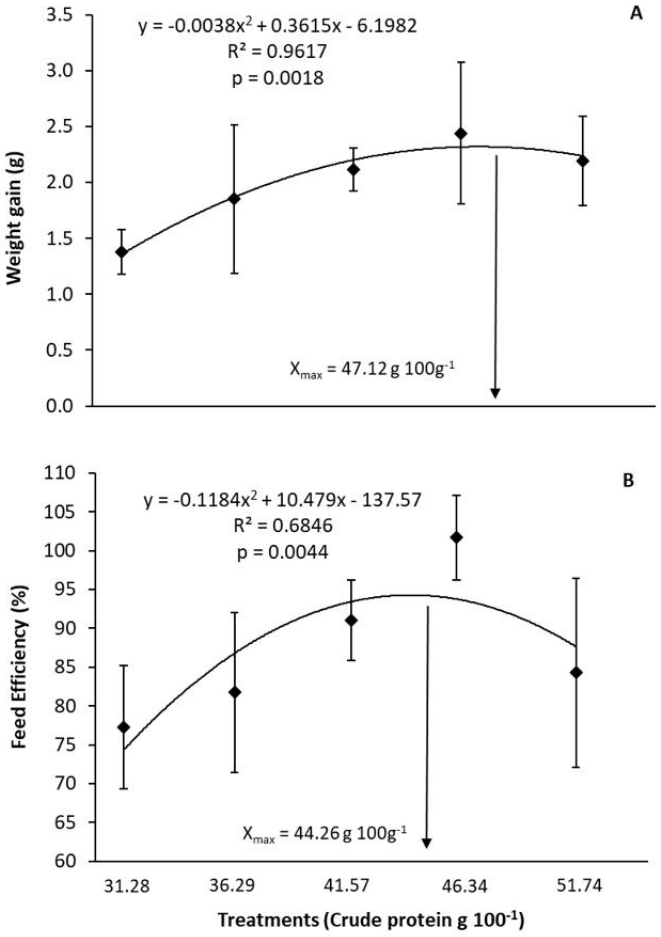

Figure 1. Polynomial regression of (A) weight gain (Protein requirement (95% of X_{max}) = 44.76 g 100 g^{-1}) and (B) feeding efficiency (Protein requirement (95% of X_{max}) = 42.05 g 100 g^{-1}) of the Pacific white shrimp *Litopenaeus vannamei* raised for 38 days in a biofloc system, using diets with different levels of crude protein. Coefficients are significant at 5% probability by analysis of variance. n = 3

is consistent with that of Hari et al. (2006) in a study of *Penaeus monodon*, in which it was necessary to apply an organic carbon source to the 40 g 100 g^{-1} CP and 25 g 100 g^{-1} CP treatments to maintain similar levels of total nitrogen.

Ebeling et al. (2006) and Hargreaves (2013) recommend $CaCO_3$ concentrations between 100 and 150 mg L^{-1}. In this work, the alkalinity was higher than 140 mg L^{-1} in all treatments, adding calcium to hydrate the system. The amount of calcium generally increased, along with the protein content of the diet. This may be related to the oxidation of nitrogenous compounds, which consume carbonates and bicarbonates, essential nutrients for the development of nitrifying bacteria and hardening of shrimp carapace (Ebeling et al., 2006).

Composition of shrimp and biofloc sludge

In this study, the composition of shrimp and biofloc sludge was not influenced by the protein content, although the shrimp fed diets with high crude protein content tended to have a higher crude protein content, ranging from 65.29% to 72.83%, as well as increasing ethereal extract (10.45–11.65% EE) and nitrogen (10.45–11.64% N). Similar trends were observed in the crude protein and nitrogen content of the biofloc sludge with treatments of 36.29 g 100 g^{-1} and above.

In biofloc systems, Jatobá et al. (2014) reported that the using diets containing different protein levels (32.9 and 36.7% CP) the shrimp had higher energy content in their tissue, but in the other assessments (protein, crude lipid, crude fiber, and minerals), there were no significant differences in bioflocs or the shrimp, similar to the results found in the present study. In the nursery phase (weight initial ~0.01 g), the bioflocs contributed 22–43% C and 0–43% N to shrimp tissue composition, whereas in the grow-out phase (weight initial ~0,83 g), C and N of biofloc origin contributed 63–100% and 35–86%, respectively. According to the same authors, the contribution of bioflocs as a supplementary source of natural food depends on the size of the shrimp Krummenauer et al. (2020) and the nutritional characteristics of bioflocs (Emerenciano et al., 2013).

Table 6. Growth performance (mean ± standard deviation) for Pacific white shrimp *Litopenaeus vannamei* post-larvae raised for 38 days in a biofloc system containing different levels of crude protein (CP) (initial weight: 0.16g) (n = 3).

Productive variables	Crude proteing 100 g^{-1}					Squared effect
	31.28	36.29	41.57	46.34	51.74	
Final weight (g)	1.5±0.2*	2.0±0.7	2.3±0.2	2.6±0.6	2.4±0.4	$y = -0.003x^2 + 0.302x - 5.035$ $R^2 = 0.951$ $p = 0.0016$
Survival (%)	93.9±8.6	90.4±8.9	92.6±4.8	94.4±7.9	84.6±12.3	Not significant
Gain in biomass (g)	248.9±63.3	292.6±76.1	350.0±24.8	409.9±68.9	327.6±68.1	$y = -0.514x^2 + 49.05x - 802.9$ $R^2 = 0.761$ $p = 0.007$
Yield (kg m^{-3})	0.7±0.2	0.8±0.2	1.0±0.1	1.1±0.2	0.9±0.2	$y = -0.001x^2 + 0.124x - 1.974$ $R^2 = 0.763$ $p = 0.006$

Growth performance

Yield reached 1.10 kg m^{-3} (at 46.34 g 100 g^{-1} CP), which is consistent with the results of Serra et al. (2015), who recorded a productivity of 1.23 kg m^{-3} at a density of 300 PL m^{-2}. However, Zhang (2011) and Legarda et al. (2018) found higher rates of productivity compared to those recorded in the present study, although these results appear to be related to the higher stocking rates adopted in these studies.

The survival was above 80%, with no significant variation among treatments, because of the excellent growth performance typical of this phase in the rearing process (Wasielesky et al., 2006; Mishra et al., 2008; Maicá et al., 2012; Khanjani et al., 2016).

The values recorded for feeding efficiency in the present study (77.28–101.68%) were like those found in studies of *L. vannamei* nurseries based on biofloc systems, which reported a mean efficiency of 81 to 95% (Widanarni et al., 2010; Khanjani et al., 2016).

In the present study, the optimum rates of growth and conversion of ration into animal tissue (feeding efficiency) were recorded for the 46.34 g 100 g^{-1} CP treatment. Maximum weight gain was recorded for the diet with a crude protein content of 47.12 g 100 g^{-1}, although maximum feeding efficiency was estimated to occur at 44.26 g 100 g^{-1} CP (Figure 1). Therefore, the adoption of a diet with crude protein content between 44.26 and 47.12 g 100 g^{-1}would support the best performance in juvenile *L. vannamei*.

In the present study, the decreasing yield trends observed in the 51.74 g 100 g^{-1} CP treatment, together with the estimated interval of optimal performance, indicate that this crude protein content exceeds the dietary requirements of the study species. In this case, it is plausible that the excess of amino acids was catabolized to fulfill the energetic requirements of the animal, rather than the repair or synthesis of new tissue, with the excess excreted in the form of ammonia (Shiau, 1998; Gatlin III, 2010; NRC, 2011; Portz and Furuya, 2013). However, the lower protein content of the diet of biofloc systems is also reflected in a reduction in the growth performance of the shrimp, as shown by Jatobá et al. (2014) in a study of *L. vannamei* reared in a biofloc system.

CONCLUSION

The protein requirement for the initial phase of rearing Pacific white shrimp *Litopenaeus vannamei* in biofloc is between 42.05 and 44.76 g 100 g^{-1}, considering shrimp weight gain and feed conversion ratio respectively.

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