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APPLIED STUDIES

Crude protein in low-fish meal diets for juvenile *Litopenaeus vannamei* can be reduced through a well-balanced supplementation of essential amino acids

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The use of supplemental essential amino acids (EAAs) has been shown to provide an opportunity to minimize excess levels of crude protein (CP) in animal feeds. The present study investigated the effect of reducing the amount of CP in low-fish meal diets (5%) for juvenile *Litopenaeus vannamei*. Four sets of diets were prepared containing (% on a fed basis, mean ± *SD*) 31.24 ± 0.71, 33.70 ± 0.41, 36.90 ± 0.44, and 39.63 ± 0.14% CP, with each protein level containing a total dietary methionine (Met) (Met + Cys) level of 0.56 ± 0.02 (1.07 ± 0.23), 0.71 ± 0.01 (1.22 ± 0.20), 0.88 ± 0.02 (1.38 ± 0.22), or 1.04 ± 0.02% (1.55 ± 0.18%). Shrimp of 1.00 ± 0.08 g were stocked in 84 outdoor tanks of 1 m^3 at a rate of 100 shrimp/m^2 and raised for 75 days. Final survival ranged from 83 to 97% and was unaffected by Met content. Both survival and yield were significantly depressed when shrimp were fed the 31% CP diet. Shrimp grew at a weekly rate of between 0.79 and 0.97 g, achieving a final body weight (BW) in excess of 10.8 g. There was a significant interaction between CP and Met over BW. Shrimp fed 0.56% Met achieved the lowest BW at harvest. Increasing CP beyond 34% did not enhance BW. With a dietary Met content of 0.71%, the highest BW was achieved with 34% CP compared to other levels. There was a significant improvement in Feed Conversion Ratio (FCR) when CP was raised from 31 to 34%. Similarly, dietary Met levels above 0.71% resulted in a significantly better FCR compared to 0.56%. Our study has shown that, if dietary Met (Met + Cys) meets a minimum of 0.71% (1.22%), levels of CP could be reduced from 40 to 34% without adverse effects on shrimp performance.

KEYWORDS

amino acid, DL-methionyl-DL-methionine, protein, requirement

1 | INTRODUCTION

In aquaculture, both protein quantity and quality are given importance to achieve optimal protein deposition and growth of animals. Amino acid (AA) balance in relation to the requirements of the animal determines protein quality, and a diet with a balanced AA profile reduces AA catabolism and increases protein retention. Meeting the requirements of all AAs via intact protein sources often results in excessive amounts of dietary protein levels. Inputs of protein represent a significant cost to shrimp formulas. As a consequence, several nutritional strategies have been attempted to spare dietary protein (lipid–protein ratio, Hu et al., 2008; synthetic AAs, Huai et al., 2010; carbohydrates, Wang et al., 2015; bioflocs, Yun et al., 2016).

A dietary content of 30% of digestible protein (% of the diet, dry matter basis, DM), balanced for AAs, is recommended to be optimal for juvenile whiteleg shrimp *Litopenaeus vannamei* (NRC, 2011). However, crude protein (CP) values in industrially compounded feeds generally exceed those advocated by NRC (2011). Recently, Chatvijitkul, Boyd, and Davis (2018) reported CP levels between 33 and 43% (nitrogen × 6.25, DM) in 39 samples of whiteleg shrimp feeds manufactured in Asia and the Americas. Formulators still meet the bulk of shrimp requirements for essential amino acids (EAAs) through protein-bound sources (Nunes, Sá, Browdy, & Vázquez-Añón, 2014). This results in feeds containing protein levels above the needs of the animal. There is also an indigestible portion of the protein invariably present in practical feedstuffs, which raises the total dietary CP content. Nevertheless, excess protein is catabolized by shrimp and released as ammonium compounds. This can build up in the culture water and soil, resulting in problems with water quality and, ultimately, disease outbreaks (Samocha et al., 2017). The use of the ideal protein concept in formulating diets to meet the requirements of EAAs with no deficiency and no or minimal excess has the potential to spare the dietary CP content by supplementing indispensable AAs through synthetic sources.

Our recent studies showed that Met required in practical diets for the maximum growth of juvenile whiteleg shrimp varied from 0.80 to 0.94% (1.26–1.40% methionine (Met) + cysteine) depending on the culture system (outdoor static versus flow-through system, 70 shrimp/m^2; Façanha, Sabry-Neto, Figueiredo-Silva, Oliveira-Neto, & Nunes, 2018) and from 0.72 to 0.81% (1.19–1.28% Met + Cys) depending on stocking density (50–75 shrimp/m^2; Façanha, Oliveira-Neto, Figueiredo-Silva, & Nunes, 2016). However, given that Met is the first limiting AA in shrimp feed, it is important to further strengthen our knowledge on this subject.

The present study investigated the combined effect of reducing CP with graded levels of Met in a low-fish meal diet on the growth performance and feed efficiency of juvenile *L. vannamei*. CP was reduced through a well-balanced supplementation of crystalline amino acids (CAAs).

2 | MATERIALS AND METHODS

2.1 | Experimental design

The study diets were formulated to contain graded levels of CP and dietary Met. Dietary CP varied from 31 to 40% (% of the diet, as is basis) with a corresponding Met level between 0.6 and 1.0% (% of the diet, as is). Juvenile *L. vannamei* were stocked in 84 outdoor tanks of 1 m^3 at a rate of 100 shrimp/m^2 (102 shrimp/tank). Five rearing tanks were randomly assigned to each combination of dietary CP and Met level. Shrimp were fed 10 times daily with an automatic feeding device over a continuous 75-day rearing period. At harvest, shrimp were counted and weighed on an electronic scale, and their survival, growth performance, yield, and feed efficiency were determined.

2.2 | Diets

A total of 16 diets were formulated and split into four sets of CP (31, 34, 37, and 40%, as is) and Met content (0.56, 0.71, 0.88, and 1.04%, as is; Table 1) in a 4 × 4 factorial design. Dietary CP was increased by higher inclusion levels

TABLE 1 Ingredient and proximate composition of diets used in this study

Dietary	Ingredient composition (% of the diet, as is basis)																
Protein (%)	**31**				**34**				**37**				**40**				
Methionine (%)	**0.56**	**0.71**	**0.88**	**1.04**	**0.56**	**0.71**	**0.88**	**1.04**	**0.56**	**0.71**	**0.88**	**1.04**	**0.56**	**0.71**	**0.88**	**1.04**	**CTL**
Wheat flour[1]	38.47	38.76	38.89	39.02	33.31	33.44	33.56	33.69	28.40	28.53	28.61	28.59	21.96	22.35	22.33	22.33	–
Soybean meal[2]	14.80	14.80	14.80	14.80	19.35	19.35	19.35	19.35	23.69	23.69	23.69	23.69	27.73	27.73	27.73	27.73	–
SPC[3]	11.74	11.50	11.28	11.05	15.56	15.34	15.13	14.91	18.80	18.58	18.38	18.20	21.46	21.30	21.15	21.00	–
Vital wheat gluten[4]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	–
Salmon meal[5]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	–
Cassava starch	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	–
Salmon oil	3.01	3.01	3.01	3.01	3.01	3.01	3.01	3.00	3.01	3.01	3.01	3.01	3.01	3.01	3.01	3.01	–
Soy lecithin	3.00	3.00	3.00	3.00	2.89	2.89	2.89	2.89	2.92	2.92	2.92	2.91	2.88	2.88	2.88	2.88	–
Yellow kaolin	2.65	2.39	2.26	2.13	1.51	1.39	1.26	1.14	0.22	0.09	–	–	–	–	–	–	–
Krill meal[6]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	–
Whole squid meal[7]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	–
MSP[8]	1.78	1.78	1.78	1.79	1.66	1.66	1.66	1.66	1.60	1.60	1.60	1.60	1.67	1.67	1.68	1.68	–
L-Lysine[9]	0.93	0.95	0.96	0.97	0.45	0.46	0.48	0.49	0.02	0.03	0.04	0.06	–	–	–	–	–
Magnesium sulfate	1.27	1.28	1.29	1.30	1.14	1.14	1.15	1.16	1.02	1.03	1.04	1.05	0.93	0.80	0.80	0.80	–
Soybean oil	1.24	1.24	1.25	1.25	1.26	1.27	1.27	1.28	1.16	1.17	1.18	1.18	1.17	1.08	1.08	1.08	–
Calcium carbonate	1.18	1.18	1.18	1.19	1.11	1.11	1.11	1.11	1.04	1.04	1.05	1.05	1.01	0.99	0.99	0.99	–
L-Arginine[10]	0.46	0.47	0.48	0.49	0.15	0.16	0.17	0.18	–	0.02	0.03	0.04	–	–	–	–	–
Vit.–min. premix[11]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–
Potassium chloride	0.57	0.57	0.58	0.59	0.29	0.30	0.30	0.31	0.04	0.05	0.06	0.06	0.11	–	–	–	
L-Threonine[12]	0.32	0.33	0.33	0.34	0.16	0.16	0.17	0.17	0.01	0.01	0.02	0.02	–	–	–	–	–
Binder[13]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	–
L-Trytophan[14]	0.07	0.07	0.07	0.07	0.02	0.02	0.02	0.02	–	–	–	–	–	–	–	–	–
DL-Met-Met[15]	0.10	0.26	0.42	0.58	0.04	0.20	0.36	0.52	–	0.16	0.32	0.47	–	0.12	0.28	0.43	–
L-Phenylalanine[16]	0.20	0.20	0.21	0.21	–	–	–	–	–	–	–	–	–	–	–	–	–
L-Histidine[17]	0.13	0.13	0.14	0.14	0.02	0.03	0.03	0.03	–	–	–	–	–	–	–	–	–
Cholesterol[18]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	–

TABLE 1 (Continued)

Dietary	Ingredient composition (% of the diet, as is basis)																
Protein (%)	31				34				37				40				
Methionine (%)	0.56	0.71	0.88	1.04	0.56	0.71	0.88	1.04	0.56	0.71	0.88	1.04	0.56	0.71	0.88	1.04	CTL
Ascorbic acid[19]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	—
Proximate composition (% of the diet, as is)[20]																	
Dry matter	90.69	90.49	90.53	91.33	91.95	90.83	89.32	91.72	90.62	90.19	91.73	91.24	90.55	91.61	90.88	90.24	91.19
Crude protein	30.56	31.19	30.98	32.22	33.98	33.83	33.10	33.90	36.65	36.45	37.43	37.06	39.65	39.67	39.43	39.76	36.45
Ether extract	7.30	7.62	8.44	8.68	7.81	8.18	7.78	8.91	8.31	8.28	8.69	8.02	7.59	8.76	8.75	9.18	9.48
Crude fiber	1.89	1.90	2.11	1.90	2.58	2.67	2.58	1.97	2.63	2.48	2.43	2.53	2.17	2.32	2.55	3.68	3.37
Crude ash	9.39	9.14	9.14	9.42	8.84	8.43	8.30	8.40	7.43	7.34	7.06	7.16	7.38	7.36	7.25	7.40	12.22
NFE[21]	41.55	40.64	39.86	39.11	38.74	37.72	37.56	38.54	35.60	35.64	36.12	36.47	33.76	33.50	32.90	30.22	29.67
GE (MJ/kg)[22]	19.07	19.21	19.37	19.47	19.49	19.61	19.47	19.80	19.96	19.94	20.18	19.98	19.98	20.27	20.25	20.31	19.41

[1] 9.21% crude protein (CP, as is basis), 0.22% lysine (Lys), 0.15% methionine (Met), 0.37% methionine + cystine (Met + Cys), 0.26% threonine (Thr), 0.37% arginine (Arg).

[2] Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil). 48.80% CP, 2.87% Lys, 0.62% Met, 1.33% Met + Cys, 1.83% Thr, 3.49% Arg.

[3] Sementes Selecta S.A. (Araguari, Goiás, Brazil). 61.64% CP, 3.76% Lys, 0.80% Met, 1.62% Met + Cys, 2.45% Thr, 4.56% Arg.

[4] Amytex 100. Tereos Syral S.A.S. (Marckolsheim, France). 78.71% CP, 1.37% Lys, 1.22% Met, 2.80% Met + Cys, 2.01% Thr, 2.84% Arg.

[5] Pesquera Pacific-Star (Puerto Montt, Chile). 59.38% CP, 4.15% Lys, 1.56% Met, 2.06% Met + Cys, 2.44% Thr, 3.58% Arg.

[6] Qrill™ Antarctic krill meal, Aker BioMarine Antarctic AS (Oslo, Norway). 57.05% CP, 3.92% Lys, 1.63% Met, 2.08% Met + Cys, 2.54% Thr, 3.45% Arg.

[7] 83.13% CP, 5.06% Lys, 2.34% Met, 3.17% Met + Cys, 3.33% Thr, 5.69% Arg.

[8] Monosodium phosphate.

[9] Biolys®, L-Lysine 54.6%. Evonik Nutrition and Care GmbH (Hanau, Germany).

[10] L-Arginine HCl 99% (Sigma-Aldrich do Brasil Ltda., São Paulo, Brazil).

[11] 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; vit. D3, 350,000 IU; vit. E, 25,000 IU; vit. K3, 500 mg; vit. B1, 5,000 mg; vit. B2, 4,000 mg; vit. B6; 10 mg; nicotinic acid, 15,000 mg; pantothenic acid, 10,000 mg; biotin, 150 mg; folic acid, 1,250 mg; vit. C, 25,000 mg; choline, 50,000 mg; inositol, 20,000 mg; Fe 2,000 mg; Cu, 3,500 mg; chelated Cu, 1,500 mg; Zn, 10,500 mg; chelated Zn, 4,500 mg; Mn, 4,000 mg; Se, 15 mg; chelated Se, 15 mg; I, 150 mg; Co, 30 mg; Cr, 80 mg; filler, 1,000 g.

[12] ThreAMINO®, L-Threonine, Feed Grade 98.5%. Evonik Nutrition and Care GmbH.

[13] Nutri-Bind Aqua Veg Dry, Nutri-Ad International NV (Dendermonde, Belgium). Synthetic pellet binder consisting of calcium lignosulfonate (94.00%) and guar gum (6.00%).

[14] TrypAMINO®, L-Tryptophan, Feed Grade 98%. Evonik Nutrition and Care GmbH.

[15] AQUAVI® Met-Met, DL-Methionyl-DL-Methionine 95%. Evonik Nutrition and Care GmbH.

[16] L-Phenylalanine, 98.5%. Courtesy of Evonik Nutrition and Care GmbH.

[17] L-Histidine, 98.5%. Courtesy of Evonik Nutrition and Care GmbH.

[18] Cholesterol SF, minimum of 91% of active cholesterol; Dishman Netherlands B.V. (Veenendaal, Netherlands).

[19] Rovimix® Stay C® 35. Minimum of 35% of phosphorylated vitamin C activity. DSM Nutritional Products AG (Schweiz, Switzerland).

[20] Analyses carried out by CBO Análises Laboratoriais (Valinhos, São Paulo, Brazil). Crude protein analyzed by Evonik Nutrition and Care GmbH.

[21] Nitrogen-free extract. Calculated by difference: [dry matter – (crude protein + fat + crude fiber + ash)].

[22] Gross energy (GE, MJ/kg) given on a dry matter (DM) basis. Calculated as GE = (4,143 + (56 × ether extract [% DM]) + (15 × crude protein [% DM]) – (44 × crude ash [% DM])) × 0.0041868. Source: Ewan (1989).

of soybean meal (SBM) and soy protein concentrate (SPC) from a mean of 14.8 and 11.4% to 27.7 and 21.2%, respectively. Inclusion levels of wheat flour, yellow kaolin, and mineral sources were modified in accordance with the amount of SBM and SPC used to balance the diets for energy and minerals. Supplemental AAs were added to balance the diets for AAs except for Met. DL-Methionyl-DL-Methionine (AQUAVI® Met-Met, Evonik Nutrition & Care GmbH, Hanau, Germany) was added at different levels to meet the targeted Met and Met + Cys levels in the diets. To ensure an adequate feed attractability, inclusion of krill meal was set at 2.5%. Dietary phospholipid was set at 2.5% and was supplied mainly by soy lecithin. Monosodium phosphate was used to meet a minimum of 0.4% of available phosphorous. Cholesterol was used to meet a minimum level of 0.10% in all diets.

Diets were analyzed for dry matter (drying in a convection oven for 24 hr at 105°C) and CP (Dumas method of nitrogen estimation) following standard methods (AOAC, 2002). Determination of AA concentration in dietary ingredients and diets was carried out following procedures described in Figueiredo-Silva, Lemme, Sangsue, and Kiriratnikom (2015). Dry matter, CP, and AA contents were analyzed in the analytical laboratory of Evonik Nutrition and Care GmbH, Germany.

Dietary CP levels (% of the diet, as is; mean ± *SD*) were determined to be 31.24 ± 0.71, 33.70 ± 0.41, 36.90 ± 0.44, and 39.63 ± 0.14% CP across the diets (Table 2). Ether extract, crude fiber, and crude ash levels were similar across diets and were determined to be 8.27 ± 0.54, 2.40 ± 0.44, and 8.09 ± 0.88%. Estimated gross energy content in the experimental diets was 19.77 ± 0.39 (MJ/kg), calculated using the equation, $\text{GE} = (4{,}143 + (56 \times \text{ether extract [\% DM]}) + (15 \times \text{CP [\% DM]}) - (44 \times \text{crude ash [\% DM]})) \times 0.0041868$, according to Ewan (1989). Nitrogen-free extract increased as dietary CP was reduced, from an average of 32.60 to 40.29%. Total dietary Met (Met + Cys) levels within each set of CP level were 0.56 ± 0.02 (1.07 ± 0.23), 0.71 ± 0.01 (1.22 ± 0.20), 0.88 ± 0.02 (1.38 ± 0.22), and 1.04 ± 0.02% (1.55 ± 0.18%) on an as-fed basis. One commercial grower shrimp feed with 36.45% CP and 0.76% Met (1.18% Met+Cys, as is) was used as a control (CTL).

Minimum amounts of EAA targeted in the experimental diets, other than that of Met, were: 1.92% Lys, 1.31% Thr, 0.37% Trp, 2.07% Arg, 0.75% His, 1.07% Iso, 1.84% Leu, 1.21% Val, and 1.47% Phe. Recommendations for AAs other than Met were based on industry knowledge, data published in recent years (Huai et al., 2009; Jin, Liu, Liu, & Zhang, 2017; Li et al., 2015; Liu et al., 2014; NRC, 2011; F. Xie et al., 2012; Zhou et al., 2012; Zhou, Wang, Wang, & Tan, 2013) and also on our past experience (Façanha et al., 2016, 2018). To balance other dietary EAAs while reducing CP content, diets were also supplemented with L-Lysine, L-Arginine, L-Threonine, L-Tryptophan, L-Phenylalanine, and L-Histidine. Diets were manufactured with laboratory equipment as described in Nunes, Sá, and Sabry-Neto (2011).

2.3 | Water stability of feed pellets

The physical stability of feed pellets in seawater was measured with a horizontal orbital shaker following the modified method of Obaldo, Divakaran, and Tacon (2002). Initially, 25 g of shrimp feed was added into a 250 mL-Erlenmeyer flask containing 100 mL of seawater at 35 g/L salinity and a temperature of 27°C. The Erlenmeyer flask was placed on the orbital shaker (Incubadora Lac-INR-1000, Láctea, São Paulo, Brazil) and set up to operate at 200 ± 15 rpm for 30 min. After this period, the feed sample was distributed on a mesh screen Tyler #20 (equivalent to 0.86 mm). Excess water in the samples was drained from the screen and washed with distilled water. The sample was dried in a convection oven for 24 hr at 130°C, and the dried sample was weighed. Water stability (%) was determined by the formula: (final weight of the feed sample adjusted for moisture content × 100)/initial weight of the feed sample (25 g). To adjust the final dry feed weight to the initial moisture content of the feed sample, final feed weight was divided by the initial feed moisture content.

2.4 | Shrimp and experimental conditions

Postlarvae (PL) of *L. vannamei* were obtained from a commercial hatchery (Aquatec Aquacultura Ltd., Canguaretama, Brazil) and nursery-reared in the lab from PL10 (postlarvae) to juvenile size. A total of 8,568 shrimp of 1.00 ± 0.08 g

TABLE 2 Amino acid profile of diets used in this study

Dietary	Amino acid composition (% of diet, as is basis)																
Protein (%)/	31				34				37				40				
Methionine (%)	0.56	0.71	0.88	1.04	0.56	0.71	0.88	1.04	0.56	0.71	0.88	1.04	0.56	0.71	0.88	1.04	CTL
Essential amino acids (EAAs)																	
Arginine	2.07	2.11	2.08	2.18	2.18	2.16	2.12	2.16	2.30	2.31	2.36	2.35	2.57	2.57	2.55	2.54	2.24
Histidine	0.63	0.64	0.63	0.67	0.74	0.73	0.72	0.73	0.82	0.82	0.83	0.82	0.90	0.89	0.90	0.90	0.87
Isoleucine	1.18	1.19	1.18	1.22	1.41	1.39	1.37	1.37	1.57	1.55	1.59	1.57	1.73	1.71	1.70	1.71	1.16
Leucine	1.98	2.01	1.99	2.07	2.35	2.33	2.29	2.32	2.62	2.61	2.65	2.62	2.86	2.85	2.83	2.84	2.56
Lysine	1.83	1.86	1.84	1.95	1.88	1.86	1.80	1.88	1.84	1.81	1.90	1.88	2.05	2.05	2.03	2.04	1.94
Methionine	0.54	0.72	0.86	1.06	0.56	0.71	0.89	1.04	0.55	0.72	0.90	1.05	0.59	0.70	0.87	1.02	0.76
Met + Cys	0.97	1.16	1.28	1.51	1.05	1.20	1.37	1.52	1.09	1.25	1.44	1.58	1.17	1.28	1.44	1.58	1.18
Phenylalanine	1.49	1.53	1.52	1.58	1.56	1.54	1.51	1.54	1.74	1.73	1.75	1.73	1.90	1.90	1.89	1.88	1.59
Threonine	1.28	1.32	1.30	1.36	1.32	1.31	1.30	1.32	1.33	1.32	1.34	1.33	1.44	1.43	1.43	1.42	1.31
Valine	1.25	1.27	1.27	1.32	1.49	1.49	1.46	1.45	1.66	1.64	1.67	1.66	1.81	1.79	1.78	1.79	1.73
Sum EAAs	12.24	12.66	12.67	13.41	13.48	13.51	13.44	13.81	14.43	14.50	15.00	15.01	15.84	15.89	15.96	16.15	14.18
Nonessential amino acids (NEAAs)																	
Alanine	1.17	1.20	1.18	1.23	1.38	1.39	1.35	1.37	1.55	1.53	1.56	1.54	1.67	1.68	1.66	1.66	2.13
Aspartate	2.48	2.55	2.51	2.60	3.07	3.04	2.99	3.03	3.51	3.51	3.56	3.53	3.92	3.91	3.89	3.89	3.21
Cystine	0.43	0.44	0.43	0.45	0.49	0.49	0.48	0.48	0.53	0.53	0.55	0.53	0.58	0.57	0.57	0.57	0.41
Glycine	1.29	1.31	1.30	1.36	1.49	1.50	1.46	1.48	1.65	1.63	1.66	1.64	1.77	1.77	1.76	1.76	2.80
Glutamate	5.66	5.78	5.74	5.96	6.49	6.44	6.32	6.41	7.06	7.02	7.12	7.07	7.50	7.49	7.44	7.44	5.35
Proline	1.74	1.76	1.79	1.84	2.00	1.91	1.96	1.92	2.07	2.10	2.13	2.07	2.19	2.20	2.13	2.19	2.20
Serine	1.30	1.34	1.32	1.36	1.55	1.53	1.50	1.54	1.74	1.73	1.76	1.74	1.89	1.90	1.88	1.87	1.61
Sum NEAA	14.07	14.37	14.27	14.78	16.47	16.30	16.05	16.23	18.10	18.05	18.32	18.11	19.51	19.52	19.33	19.38	17.71
EAA + NEAA	26.31	27.02	26.93	28.19	29.95	29.81	29.49	30.04	32.54	32.55	33.32	33.12	35.34	35.40	35.29	35.52	31.89
Free AAs																	
Methionine	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.23
Lysine	0.41	0.41	0.41	0.45	0.22	0.22	0.20	0.24	0.03	0.03	0.04	0.04	<0.02	<0.02	<0.02	<0.02	<0.02

TABLE 2 (Continued)

Dietary	Amino acid composition (% of diet, as is basis)																
Protein (%)/	**31**				**34**				**37**				**40**				
Methionine (%)	**0.56**	**0.71**	**0.88**	**1.04**	**0.56**	**0.71**	**0.88**	**1.04**	**0.56**	**0.71**	**0.88**	**1.04**	**0.56**	**0.71**	**0.88**	**1.04**	**CTL**
Threonine	0.28	0.28	0.29	0.31	0.16	0.16	0.15	0.17	0.02	0.02	0.03	0.03	<0.01	<0.01	<0.01	0.02	<0.01
Valine	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Arginine	0.38	0.39	0.39	0.43	0.18	0.18	0.17	0.20	0.07	0.08	0.10	0.10	0.08	0.08	0.08	0.08	0.06
Histidine	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Phenylalanine	0.17	0.17	0.17	0.19	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
DL-Met-Met	0.07	0.20	0.31	0.45	0.03	0.16	0.26	0.42	<0.01	0.10	0.26	0.39	<0.01	0.09	0.22	0.30	<0.01

TSAA: total sulfur amino acids.

(*CV* = 7.5%) were individually weighed for culling. Shrimp were stocked in outdoor tanks of 1 m^3 with a bottom surface area of 1.02 m^2. Five rearing tanks were randomly assigned for each set of the experimental diets. Four tanks were assigned for the CTL. The rearing system and water preparation were the same as that adopted by Nunes et al. (2011) and Façanha et al. (2016, 2018).

During shrimp culture, dried sugarcane molasses was applied as a carbon source to stimulate microbial growth and limit water exchange to a minimum (Samocha et al., 2007). Molasses was administered over the water surface once or twice a week at 5 g/m^3. Whenever required, freshwater was added to control increments in water salinity. Tanks were provided with continuous aeration to reach near saturation of dissolved oxygen. Water temperature, salinity, and pH remained relatively stable during culture at $29.8 \pm 1.33°C$ (n = 4,366), 34 ± 3.1 g/L (n = 4,366), and 8.00 ± 0.28 (n = 4,366), respectively.

Shrimp were fed following a feeding table. Feeding rates were calculated based on the maximum amount of feed (MM, g) that can be eaten daily by one individual of a specific body weight (BW), in accordance with the formula $MM = 0.0931BW^{0.6200}$ (Façanha et al., 2018; Nunes & Parsons, 2000; Nunes, Sá, Carvalho, & Sabry-Neto, 2006). To avoid excess feeding and a high feed conversion ratio (FCR), feeding rates were reduced by 20% across all diets.

Diets were delivered 10 times a day using a feeding dispenser device, which operated during 10-hr periods, between 07:30 a.m. and 05:30 p.m. In the first 12 days of rearing, meals were adjusted on a daily basis following an estimated weight gain of 100 mg day^{-1} $shrimp^{-1}$ and a 0.38% weekly drop in shrimp survival across all diets. On a biweekly (Days 13, 27, 41, 55, and 69 of rearing) basis, rations were corrected by weighing five animals per tank individually. Until the next weight check, feed ration was adjusted according to calculated daily weight of shrimp gains for each tank, maintaining a consistent reduction in survival. No dead animals were replaced throughout the culture period.

2.5 | Shrimp performance

Shrimp growth performance was assessed by counting and individually weighing each animal at stocking and harvest. Shrimp final survival (S, %) was calculated using the equation: $S = (POPf/POPi) \times 100$, where POPi = number of shrimp at stocking, and POPf = number of shrimp at harvest. Weekly weight gain (WWG, g/week) was determined by the formula: $WWG = [(Wf - Wi)/t] \times 7$, in which Wi = shrimp wet body weight (BW, g) at stocking, Wi = shrimp BW (g) at harvest, and t = number of days in culture. Gained shrimp yield (YIE, g of shrimp biomass gained/m^2) was determined using YIE = (BIOf − BIOi)/tank bottom area (m^2), where BIOi = initial shrimp biomass per tank (g), BIOf = final shrimp biomass per tank (g), and tank bottom area = 1.02 m^2. FCR was calculated on a DM basis by dividing the total amount of feed delivered (g) during culture by the wet shrimp biomass gained (g) in each tank. Apparent feed intake (AFI, g of feed delivered divided by the number of stocked shrimp) was also expressed on a DM basis.

2.6 | Statistical analysis

Statistical analyses were performed with the Statistical Package for Social Sciences, package 23 (IBM® SPSS® Statistics, Chicago, IL). The effect of dietary CP level and Met content and their interaction over shrimp performance were analyzed through two-way ANOVA. When significant differences were detected, they were compared two by two using Tukey's honestly significant difference (HSD) test. Student t-test was used to compare the differences in shrimp performance within each dietary CP level and Met content versus the CTL diet.

Homogeneity of variance was examined for all data by using the Bartlett-Box F and Cochran's C tests. Kurtosis and skewness and their *SE* (i.e., *SE* kurtosis and *SE* skewness) were applied to the data as measures of asymmetry and tests of normality. When required, data were transformed to a $\log(x + 1)$ scale in order to normalize and homogenize the variances and to meet statistical assumptions. The probability of Type I error was set at 0.05.

3 | RESULTS

Data analyses showed that CP levels affected survival, final BW, yield, and FCR, while Met level affected final BW, yield, and FCR (Table 3; Figure 1). Except for final BW, no interaction effects were detected in other response variables measured because of Met and CP levels.

Final shrimp survival ranged from 83.3 (31/0.56, CP/Met) to 96.7% (37/1.04, CP/Met) across dietary groups. Survival did not differ statistically between shrimp fed the CTL and experimental diets ($p > 0.05$). However, reducing dietary CP from 40 and 37% to 31% negatively affected survival ($p < 0.05$). On the other hand, survival was unaffected by the dietary Met content ($p > 0.05$).

Shrimp yield responded negatively to lower dietary levels of CP and Met, varying from 690 ± 116 (31/0.56, CP/Met) to 907 ± 62 g/m^2 (40/0.71, CP/Met). Yield was significantly depressed when shrimp were fed the 31% CP diet. Similarly, diets containing 0.56% Met also resulted in a statistically lower yield compared to 0.71 and 1.04% Met, although it did not differ from 0.88% Met. No significant interaction could be observed between dietary CP and Met. Gained yield for shrimp fed the CTL was lower than shrimp fed 34% CP at all Met levels. Similarly, yield was higher for shrimp fed 40% CP at 0.71, 0.88, and 1.04% Met than the CTL. Gained yield did not differ statistically between the CTL and the 31% CP diet with 0.56 and 0.88% Met and 40% CP with 0.56% Met.

AFI was not affected by dietary CP or Met content ($p < 0.05$). However, FCR improved significantly with higher levels of CP and Met, particularly at 34% CP and 0.71% Met and beyond. There was no statistical difference in FCR when shrimp were fed diets containing between 34 and 40% CP and between 0.71 and 1.04% Met.

Shrimp grew at a weekly rate of between 0.79 and 0.97 g when fed the experimental diets, while those fed the CTL achieved a mean of 0.70 ± 0.02 g ($p < 0.05$). There was some trend toward higher growth when dietary CP and Met were raised. However, no statistical differences in growth could be detected between dietary CP and Met levels ($p > 0.05$). It appeared that no benefit in shrimp growth was obtained when dietary CP and Met exceeded 37 and 0.88%, respectively.

The effect of dietary CP and Met became evident for final shrimp BW (Figure 1). Shrimp fed the experimental diets achieved a significantly higher BW compared to the commercial CTL ($p < 0.05$). While shrimp fed the CTL achieved 9.06 ± 0.23 g, the lowest BW observed in shrimp fed the experimental diets was 9.48 ± 0.75 g (31/0.56). There was a significant interaction between CP and Met over shrimp BW ($p < 0.05$; two-way ANOVA). In general, the lowest level of dietary Met, that is, 0.56%, resulted in a significantly lower BW when shrimp were fed diets with 34, 37, and 40% CP. In the 34 and 37% CP diets, the highest response in BW was obtained with 0.71% Met. However, statistical analysis indicated that increasing the CP content beyond 34% did not result in a further improvement in shrimp BW ($37, 31 \neq 40 \neq 34\%$ CP; $p < 0.05$). Similarly, dietary Met levels in excess of 0.71% did not enhance shrimp BW ($0.88, 0.56 \neq 1.06 \neq 0.71\%$ Met, $p < 0.05$).

The physical water stability of feed pellets gradually dropped with a reduction in CP content (Table 4). The highest water stability was observed for diets containing 37% CP, followed by 40, 34, and 31%. Conversely, a dietary Met level of 1.04% resulted in the highest water stability compared to 0.88, 0.56, and 0.71% Met. The CTL diet showed the highest water stability compared to experimental diets evaluated.

4 | DISCUSSION

Results from the present study indicated that growth performance of juvenile *L. vannamei* fed a practical diet with only 5% fish meal can be maximized with 34% CP (nitrogen × 6.25) as long as Met (Met + Cys) levels are kept at a minimum of 0.71% (1.22%). Our value corresponds to the range of 32–36% found in several other studies carried out with juvenile *L. vannamei* (32%, Kureshy & Davis, 2002; 34%, Hu et al., 2008; 36%, Huai et al., 2010; 33%, Shahkar et al., 2014; 35%, Yun et al., 2016; 32–36%, Lee & Lee, 2018). As opposed to the present work, these CP levels were determined at the cost of a high dietary inclusion of fish meal, from 16 to 42% (16%, Kureshy & Davis,

TABLE 3 Growth performance (mean ± SD) of *Litopenaeus vannamei* after 75 days

		Dietary crude protein (%, as is)					
Variable	**% Met**	**31**	**34**	**37**	**40**	**Mean ± *SD***	**CTL**
Survival	0.56	83.3 ± 13.8A	85.9 ± 7.02A	90.4 ± 7.13A	93.7 ± 2.56A	88.2 ± 9.00	—
	0.71	86.1 ± 8.16A	87.8 ± 7.80A	92.5 ± 2.26A	94.9 ± 6.74A	90.3 ± 7.12	—
	0.88	84.9 ± 9.77A	96.5 ± 2.15B	95.1 ± 3.47AB	92.7 ± 5.08AB	92.3 ± 7.08	—
	1.04	92.9 ± 4.01A	91.4 ± 5.94A	96.7 ± 1.78A	92.9 ± 7.44A	93.5 ± 5.05	93.1 ± 2.65
	Mean ± *SD*	86.8 ± 9.58A	90.4 ± 6.97AB	93.9 ± 4.32B	93.6 ± 5.22B	—	—
Gained yield (g/m^2)	0.56	690 ± 116aA	859 ± 79aB*	839 ± 71aAB*	820 ± 69aAB	800 ± 105a	—
	0.71	817 ± 39abA*	894 ± 59aA*	887 ± 53aA*	907 ± 62aA*	876 ± 61b	—
	0.88	786 ± 55abA	856 ± 40aA*	834 ± 91aA*	883 ± 38aA*	840 ± 66ab	—
	1.04	866 ± 45bA*	878 ± 54aA*	862 ± 97aA*	887 ± 28aA*	872 ± 59b	743 ± 14*
	Mean ± *SD*	790 ± 93A	872 ± 57B	857 ± 77B	874 ± 60B	—	—
Growth (g/day)	0.56	0.79 ± 0.07*	0.95 ± 0.04*	0.88 ± 0.14*	0.82 ± 0.05*	0.86 ± 0.10	—
	0.71	0.91 ± 0.08*	0.97 ± 0.10*	0.90 ± 0.06*	0.90 ± 0.08*	0.92 ± 0.08	—
	0.88	0.89 ± 0.13*	0.83 ± 0.06*	0.83 ± 0.11	0.90 ± 0.02*	0.86 ± 0.09	—
	1.04	0.88 ± 0.07*	0.91 ± 0.04*	0.84 ± 0.10*	0.91 ± 0.11*	0.88 ± 0.08	0.70 ± 0.02*
	Mean ± *SD*	0.87 ± 0.09	0.91 ± 0.08	0.86 ± 0.10	0.88 ± 0.07	—	—
AFI[1] (g/shrimp)	0.56	10.8 ± 0.52	11.7 ± 0.27*	11.4 ± 0.86*	11.2 ± 0.46*	11.3 ± 0.62	—
	0.71	11.2 ± 0.37*	11.7 ± 0.49*	11.3 ± 0.49*	11.5 ± 0.32*	11.4 ± 0.43	—
	0.88	11.2 ± 0.62	11.2 ± 0.39*	11.2 ± 0.64	11.5 ± 0.16*	11.3 ± 0.48	—
	1.04	11.3 ± 0.25*	11.5 ± 0.30*	11.3 ± 0.66	11.6 ± 0.33*	11.4 ± 0.40	10.7 ± 0.08*
	Mean ± *SD*	11.1 ± 0.48	11.5 ± 0.40	11.3 ± 0.61	11.5 ± 0.33	—	—
FCR[2]	0.56	1.59 ± 0.23aA	1.37 ± 0.10aB	1.36 ± 0.02aB*	1.38 ± 0.10aB	1.43 ± 0.16a	—
	0.71	1.38 ± 0.07abA	1.31 ± 0.06aA*	1.28 ± 0.08aA*	1.27 ± 0.07aA*	1.31 ± 0.43b	—
	0.88	1.43 ± 0.10abA	1.31 ± 0.04aA*	1.35 ± 0.08aA	1.31 ± 0.05aA*	1.35 ± 0.48b	—
	1.04	1.31 ± 0.05bA*	1.31 ± 0.06aA*	1.31 ± 0.08aA*	1.30 ± 0.04aA*	1.31 ± 0.41b	1.44 ± 0.04*
	Mean ± *SD*	1.42 ± 0.16A	1.32 ± 0.07B	1.32 ± 0.07B	1.32 ± 0.08B	—	—

ANOVA	**Survival**	**Yield**	**Growth**	**AFI**	**FCR**
CP	0.006	<0.0001	0.200	0.048	0.001
Met	0.104	0.003	0.108	0.698	<0.0001
CP × Met	0.445	0.380	0.183	0.481	0.272

For each response variable, values in each row and column sharing different lowercase and capital letters indicate significant differences ($p < 0.05$) because of dietary Met at the same crude protein (CP) level and because of CP level at the same Met level, respectively. When statistically significant, two-by-two comparisons were determined using Tukey's HSD test. Asterisks (*) refer to statistical difference with the CTL diet ($p < 0.05$, student *t*-test).

[1] Apparent feed intake (AFI, g) is the amount of feed delivered divided by the number of stocked shrimp.

[2] Feed conversion ratio.

2002; 30%, Hu et al., 2008; 17%, Huai et al., 2010; 34–42%, Shahkar et al., 2014; 42%, Yun et al., 2016; 18%, Lee & Lee, 2018).

Colvin and Brand (1977) were the first to evaluate optimal protein levels for *L. vannamei*. The authors formulated diets with CP levels between 25 and 40% containing 15% sun-dried shrimp meal and 15% menhaden fish meal. The increase in dietary CP was achieved by higher use of SBM at the cost of ground whole wheat. No supplementation

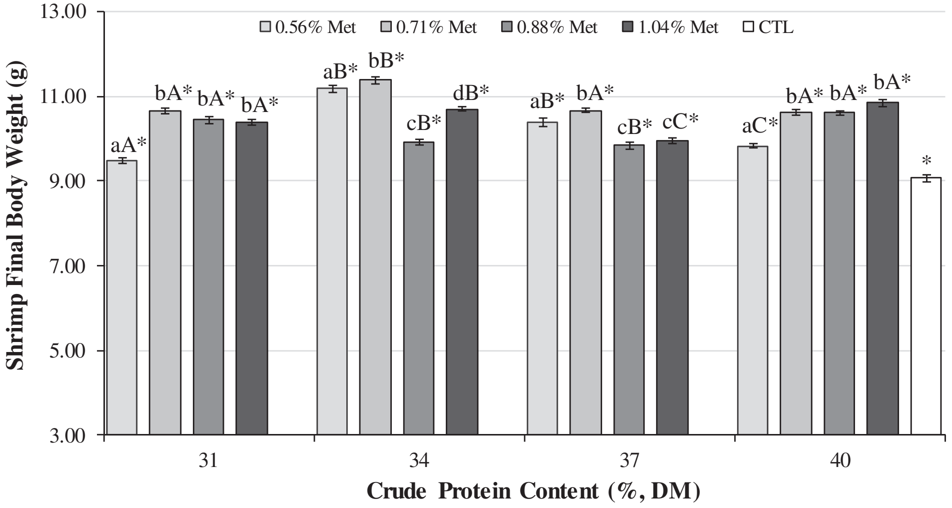

FIGURE 1 Mean (±*SE*) body weight (g) of *Litopenaeus vannamei* after 75 days of rearing in green-water tanks of 1 m^3. Different lowercase letters indicate statistically significant differences between dietary Met content within each crude protein (CP) level at the $\alpha = 0.05$ level by Tukey's HSD test. Different capital letters refer to significant differences ($p < 0.05$) between CP levels within each dietary Met. Asterisks (*) refer to statistically significant difference between the commercial control (CTL) and the experimental diets ($p < 0.05$, student t-test)

with crystalline AA was carried out. Authors reported that PL *L. vannamei* with a starting BW of 32 mg need between 30 and 35% of CP, with a further reduction below 30% in juvenile stages. Different from formulations designed by Colvin and Brand (1977), our diets contained high levels of SBM and SPC, from 44 to 50%, along with the supplemental sources of EAAs, to be the dietary CP levels. The responses of shrimp to dietary protein level were rather to meet the requirements for all EAAs in both the studies. Differences in protein digestibility, AA balance, and growth requirement between the two studies may explain the differences in the response of shrimp to the dietary protein levels.

Our findings are more in line with Smith, Lee, and Lawrence (1985) who found that 4-g *L. vannamei* and larger need a dietary CP level of more than 36% CP to meet the requirements of shrimp for all EAAs. In this case, authors increased dietary CP by higher inclusions of shrimp head meal, wheat gluten, high-protein soybean, and vitamin-free casein. In the work of Smith et al. (1985), a reduction in dietary CP likely reduced the amount of EAAs, which may

TABLE 4 Physical stability of pellets (mean ± SE) after 30 min agitation in seawater with an orbital shaker

	Dietary crude protein (%, as is)					
% Met	**31**	**34**	**37**	**40**	**Mean ± *SD***	**CTL**
0.56	66.51 ± 2.64abA*	75.19 ± 1.96aB*	86.65 ± 1.52aC*	83.98 ± 1.64abC*	78.09 ± 8.32a	93.42 ± 0.47*
0.71	70.24 ± 0.92aA*	79.99 ± 4.29aB*	87.42 ± 0.95aC*	80.75 ± 2.61aB*	79.60 ± 6.72a	—
0.88	65.07 ± 3.16bA*	78.76 ± 2.27aB*	83.32 ± 1.30bC*	84.91 ± 0.95bC*	78.01 ± 8.24a	—
1.04	54.83 ± 1.16cA*	69.28 ± 2.34bB*	84.46 ± 0.67bC*	76.97 ± 1.63cD*	71.39 ± 11.34b	—
Mean ± *SD*	64.16 ± 6.19A	75.81 ± 5.01B	85.46 ± 1.99C	81.65 ± 3.60D	—	—
Two-way	CP		Met		CP × Met	
ANOVA	<0.0001		<0.0001		<0.0001	

Values in each row and column sharing different lowercase and capital letters indicate significant differences ($p < 0.05$) because of dietary Met at the same crude protein (CP) level and because of CP level at the same Met level, respectively. When statistically significant, two-by-two comparisons were determined using Tukey's HSD test. Asterisks (*) refer to statistical difference with the CTL diet ($p < 0.05$, student t-test) have also affected the total sulfur amino acids (TSSA) content. Our study has shown that, if dietary Met (Met + Cys) meets a minimum of 0.71% (1.22%), levels of CP could be spared from 40 to 34% without adverse effects on shrimp performance. Shiau (1998) reported that estimates of protein requirement must be carefully examined because it is dependent on the quality of dietary protein (EAA profile), age, or physiological state of crustaceans. Thus, the response of shrimp to dietary protein should be assessed on the basis of dietary EAA levels, including TSSA content.

In a more recent study, Lee and Lee (2018) evaluated dietary protein levels in *L. vannamei* over three ranges of BW: 0.65 (small), 4.81 (medium), and 10.5 g (large). Authors found that the optimal dietary protein level decreases with larger BW, at 34.5, 35.6, and 32.2% for small-, medium-, and large-sized shrimp, respectively. Authors did not report the dietary AA profile, but their results corroborate our findings. Similarly, Huai et al. (2010) investigated if the CP content in *L. vannamei* diets could be decreased through supplementation with CAAs. Different from our study, authors reduced the dietary inclusion of fish meal from 22 to 17% (%, as is) to achieve a lower CP content from 41.26 and 35.52% (% of the diet, DM basis). Authors also reported no negative effects of a lower level of CP on growth performance and feed efficiency of shrimp in the BW range of 0.36 ± 0.01 to 5.95 ± 0.93 g.

In our study, a reduction in CP from 34 to 31% led to a lower shrimp survival, yield, final BW, and feed efficiency. Diets were balanced with regard to their AA profile by supplementing with CAA sources (DL-Met-Met, L-Lysine, L-Arginine, L-Threonine, L-Tryptophan, L-Phenylalanine, and L-Histidine). Therefore, the lower the amount of dietary CP, the higher the supplemental doses needed of CAAs. Comparatively, diets with higher levels of CP had an increased contribution of intact AAs and thus a lower requirement for supplemental CAAs. Lim (1993) reported that supplemental AAs can reduce the dietary pH level and that increasing dietary pH using NaOH resulted in improvements in feed intake, growth, and FCR of shrimp. In our study, we did not measure the pH of the experimental diets. However, as the protein level was reduced, a greater amount of supplemental AA was added, which could have likely reduced dietary pH. This was not reflected in feed intake data in our study. While DL-Methionyl-DL-Methionine is known to have less leaching in water and a high bioefficacy in whiteleg shrimp diets (Façanha et al., 2016, 2018; Niu et al., 2018; J.-J. Xie et al., 2018), other sources of feed-grade CAAs are likely prone to leaching in water. Pellet stability of the experimental diets was measured by examining Met levels of the diets subjected to a leaching test. We also observed that diets with reduced protein levels, that is, 31 and 34% CP, were less stable in water, which could be explained by differences in the feed composition of ingredients. As the set of 31% CP diets displayed poorer feed water stability, this could have reduced the amount of EAA available, as well as other water-soluble nutrients, at the time of shrimp feed intake. Therefore, it might have been possible to further reduce CP in whiteleg shrimp diets if feed leaching was controlled or if supplementation of CAAs was incremented to compensate for possible losses in water. The CTL diet showed the highest water stability but one of lowest growth performances. Performance was equivalent to the lowest level of CP and Met of the experimental diets tested. These results may have been driven by differences in ingredients and nutrient composition and their bioavailability and attractability.

The use of feeds containing submarginal CP levels by satisfying the required limiting EAAs is a common practice in the broiler and swine industry (Moran Jr & Stilborn, 1996; Zarate, Moran, & Burnham, 2003). Zarate et al. (2003) successfully forced a 1% reduction in CP for broiler diets, combined with a 10% increase in lysine, total sulfur amino acids (TSAA), and threonine from industry levels.

As opposed to feeds used in monogastric animal husbandry, dietary supplementation of shrimp feeds with CAAs is yet to become a widespread approach to spare CP. Instead, EAA requirements are often met with protein-bound AAs by increasing the amount of feed protein (Nunes et al., 2014). This can result in the release of excess amounts of nitrogen, causing water and soil pollution, particularly in intensive rearing systems where nitrogenous waste control becomes critical (Samocha et al., 2017). An analysis of 39 commercial shrimp feeds for the whiteleg shrimp has demonstrated that dietary CP (nitrogen × 6.25) ranges from 33 to 43% (DM basis; Chatvijitkul et al., 2018). Commercial shrimp feeds containing CP below 30% are often perceived as being of lower quality and having poor performance. However, these feeds are designed to be used in semi-intensive systems where natural food can act as an important source of nutrients to farmed shrimp. Teichert-Coddington and Rodriguez (1995) found that, under stocking densities between 5 and 11 shrimp/m^2, production performance is similar to dietary CP between 20 and 40%. In our study, the application of sugarcane molasses may have enhanced natural food availability. This could have partly contributed to shrimp CP and Met requirements. Façanha et al. (2016) have found that algal material, feed remains, and detritus (collectively called as natural food) scraped from the tank walls of shrimp-rearing tanks contained as much as 24.7% CP (on a DM basis), 0.27% Met, and 0.62% Met + Cys.

Our previous studies have shown that intensification and water exchange regime can play a critical role in the amount of the supplemental dietary Met (Met + Cys) needed to maximize whiteleg shrimp performance (Façanha et al., 2016, 2018). Higher levels of dietary Met (Met + Cys) are required in practical diets as both shrimp stocking density and water exchange are increased. In a static–water exchange conditions, only 0.80% Met (1.26% Met + Cys, DM basis) is required compared to 0.94% (1.40%) in a flow-through regime. Similarly, the requirement for dietary Met increases from 0.72 (1.19%) to 0.81% (1.28%) as shrimp density is raised from 50 to 75 shrimp/m^2. Our data are consistent with these levels, indicating that 0.78% Met (1.34% Met + Cys, DM basis) is needed to maximize shrimp performance.

Overall, our data suggest that CP content in whiteleg shrimp diets containing low-fish meal can be reduced if the EAA requirements are properly satisfied. A further reduction beyond the 34% threshold appears to be more related to an increased leaching of nutrients than to the animal's metabolic requirement. We have also found that increasing the dietary levels of dietary Met will not compensate for a lower CP content and that levels in excess of 0.71% Met (1.22% Met + Cys) are not really advantageous.

5 | CONCLUSION

From the present study, it was possible to determine that dietary CP and Met can both have an individual and combined effect over shrimp performance grown intensively under green-water conditions. Juveniles of the whiteleg shrimp can maximize their growth performance and feed efficiency when fed low-fish meal diets containing a total CP and Met (Met + Cys) content of 34 and 0.71% (1.22%), respectively. A reduction in the dietary CP from 34 to 31%, using supplemental sources to balance the diets for EAAs, leads to a poor survival, reduced yield, low BW, and increased FCR. Similarly, reducing dietary Met from 0.71 to 0.56% deteriorates FCR. Levels of dietary CP beyond 34% are not required as long as a minimum of 0.71% of Met and other EAAs are provided through a well-balanced supplementation with crystalline sources.

Under the culture conditions evaluated, shrimp survival, yield, and FCR increase, but not significantly, when dietary CP and Met ranges from 34 to 40% and from 0.71 to 1.04%, respectively. Thus, the increase in dietary CP and Met beyond 34 and 0.71%, respectively, proved unnecessary.

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REFERENCES

AOAC (Association of Official Analytical Chemists). (2002). *Official methods of analysis of the association of official analytical chemists international* (16th ed.). Arlington, VA: Author.

Chatvijitkul, S., Boyd, C. E., & Davis, D. A. (2018). Nitrogen, phosphorus, and carbon concentrations in some common aquaculture feeds. *Journal of the World Aquaculture Society*, *49*, 477–483.

Colvin, L. B., & Brand, C. W. (1977). The protein requirement of penaeid shrimp at various life cycle stages in controlled environment systems. *Proceedings of the World Mariculture Society*, *8*, 821–840.

Ewan, R. C. (1989). Predicting the energy utilization of diets and feed ingredients by pigs. In Y. van der Honing & W. H. Close (Eds.), *Energy metabolism. European Association of Animal Production bulletin no. 43* (pp. 271–274). Wageningen, Netherlands: PUDOC.

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.

Façanha, F. N., Sabry-Neto, H., Figueiredo-Silva, C., Oliveira-Neto, A. R., & Nunes, A. J. P. (2018). Minimum water exchange spares the requirement for dietary methionine for juvenile *Litopenaeus vannamei* reared under intensive outdoor conditions. *Aquaculture Research*, *49*, 1682–1689.

Figueiredo-Silva, C., Lemme, A., Sangsue, D., & Kiriratnikom, S. (2015). Effect of DL-methionine supplementation on the success of almost total replacement of fish meal with soybean meal in diets for hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*). *Aquaculture Nutrition*, *21*, 234–241.

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*, 499–506.

Huai, M.-Y., Liu, Y.-J., Tian, L.-X., Deng, S.-X., Xu, A.-L., Gao, W., & Yang, H.-J. (2010). Effect of dietary protein reduction with synthetic amino acids supplementation on growth performance, digestibility, and body composition of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture International*, *18*, 255–269.

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*, 904–914.

Jin, Y., Liu, F.-J., Liu, Y.-J., Tian, L.-X., & Zhang, Z.-H. (2017). Dietary tryptophan requirements of juvenile pacific white shrimp, *Litopenaeus vannamei* (Boone) reared in low-salinity water. *Aquaculture International*, *25*, 955–968.

Kureshy, N., & Davis, D. A. (2002). Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, *204*, 125–143.

Lee, C., & Lee, K.-J. (2018). Dietary protein requirement of Pacific white shrimp *Litopenaeus vannamei* in three different growth stages. *Fisheries and Aquatic Sciences*, *21–30*, 1–6.

Li, E., Wang, X., Chen, K., Xu, C., Qin, J. G., & Chen, L. (2015). Physiological change and nutritional requirement of Pacific white shrimp *Litopenaeus vannamei* at low salinity. *Reviews in Aquaculture*, *7*, 1–19.

Lim, C. (1993). Effect of dietary pH on amino acid utilization by shrimp (*Penaeus vannamei*). *Aquaculture*, *114*, 293–303.

Liu, F.-J., Liu, Y.-J., Tian, L.-X., Li, X.-F., Zhang, Z.-H., Yang, H.-J., & Du, Z.-Y. (2014). Quantitative dietary isoleucine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone) reared in low-salinity water. *Aquaculture International*, *22*, 1481–1497.

Moran, E. T., Jr., & Stilborn, H. L. (1996). Effect of glutamic acid on broilers given submarginal crude protein with adequate essential amino acids using feeds high and low in potassium. *Poultry Science*, *75*, 120–129.

Niu, J., Lemme, A., He, J.-Y., Li, H.-Y., Xie, S.-W., Liu, Y.-J., … Tian, L.-X. (2018). Assessing the bioavailability of the novel met-met product (AQUAVI® met-met) compared to DL-methionine (DL-met) in white shrimp (*Litopenaeus vannamei*). *Aquaculture*, *484*, 322–332.

NRC (National Research Council). (2011). *Nutrient requirements of fish and shrimp*. Washington, DC: Author.

Nunes, A. J. P., & Parsons, G. J. (2000). Size-related feeding and gastric evacuation measurements for the southern brown shrimp *Penaeus subtilis*. *Aquaculture*, *187*, 133–151.

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

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

Nunes, A. J. P., Sá, M. V. C., & Sabry-Neto, H. (2011). Growth performance of the white shrimp, *Litopenaeus vannamei*, fed on practical diets with increasing levels of the Antarctic krill meal, *Euphausia superba*, reared in clear- versus green-water culture tanks. *Aquaculture Nutrition*, *17*, 511–520.

Obaldo, L. G., Divakaran, S., & Tacon, A. G. (2002). Method for determining the physical stability of shrimp feeds in water. *Aquaculture Research*, *33*, 369–377.

Samocha, T. M., Patnaik, S., Speed, M., Ali, A.-M., Burger, J. M., Almeida, R. V., … Brock, D. L. (2007). Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering*, *36*, 184–191.

Samocha, T. M., Prangnell, D. I., Hanson, T. R., Treece, G. D., Morris, T. C., Castro, L. F., & Staresinic, N. (2017). Water quality management. In *Design and operation of super intensive, biofloc-dominated systems for indoor production of the Pacific white shrimp, Litopenaeus vannamei. The Texas a&M AgriLife research experience* (pp. 108–123). Baton Rouge, LA: The World Aquaculture Society.

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

Shiau, S. Y. (1998). Nutrient requirement of penaeid shrimps. *Aquaculture*, *164*, 77–93.

Smith, L. L., Lee, P. G., & Lawrence, A. L. (1985). Growth and digestibility by three sizes of *Penaeus vannamei* Boone: Effects of dietary protein level and protein source. *Aquaculture*, *46*, 85–96.

Teichert-Coddington, D. R., & Rodriguez, R. (1995). Semi-intensive commercial grow-out of *Penaeus vannamei* fed diets containing differing levels of crude protein during wet and dry seasons in Honduras. *Journal of the World Aquaculture Society*, *26*, 72–79.

Wang, X., Li, E., Wang, S., Qin, J., Chen, X., Lai, Q., … Chen, L. (2015). Protein-sparing effect of carbohydrate in the diet of white shrimp *Litopenaeus vannamei* at low salinity. *Aquaculture Nutrition*, *21*, 904–912.

Xie, F., Zeng, W., Zhou, Q., Wang, H., Wang, T., Zheng, C., & Wang, Y. (2012). Dietary lysine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, *358–359*, 116–121.

Xie, J.-J., Lemme, A., He, J.-Y., Yin, P., Figueiredo-Silva, C., Liu, Y.-J., … Tian, L.-X. (2018). Fishmeal levels can be successfully reduced in white shrimp (*Litopenaeus vannamei*) if supplemented with DL-methionine (DL-met) or DL-Methionyl-DL-methionine (met-met). *Aquaculture Nutrition*, *24*, 1144–1152.

Yun, H., Shahkar, E., Katya, K., Jang, I., Kim, S. K., & Bai, S. C. (2016). Effects of bioflocs on dietary protein requirement in juvenile whiteleg shrimp, *Litopenaeus vannamei*. *Aquaculture Research*, *47*, 3203–3214.

Zarate, A. J., Moran, E. T. J., & Burnham, D. J. (2003). Reducing crude protein and increasing limiting essential amino acid levels with summer-reared, slow and fast-feathering broilers. *The Journal of Applied Poultry Research*, *12*, 160–168.

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.

Zhou, Q.-C., Zeng, W.-P., Wang, H.-L., Wang, T., Wang, Y.-L., & Xie, F.-J. (2012). Dietary arginine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, *364–365*, 252–258.

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