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Feed preference and growth response of juvenile *Litopenaeus vannamei* to supplementation of marine chemoattractants in a fishmeal-challenged diet

Alberto J. P. Nunes[1] | Hassan Sabry-Neto[1] | Severino Oliveira-Neto[1] | Lena Burri[2]

[1]LABOMAR—Instituto de Ciências do Mar, Universidade Federal do Ceará, Avenida da Abolição, Fortaleza, Ceará, Brazil

[2]Aker BioMarine Antarctic AS, Lysaker, Norway

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

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Abstract

This study compared the feed preference and growth response of *Litopenaeus vannamei* to chemoattractants. A diet with 3% fishmeal was supplemented with either 3% salmon meal (POS), 3% soy protein concentrate (NEG), 3% krill meal (KRM), 3% squid meal (SQM), 3% shrimp head meal (SHM), 3% shrimp meal (SM), 3% squid liver meal (SLM), or 5% liquid sardine hydrolysate (SAH). Shrimp with a body weight (BW) of 0.99 ± 0.08 g were stocked at 100 animals/m^2 in 56 tanks of 1 m^3 and fed 10 times daily for 74 days. Feed preference was evaluated by feeding shrimp of 10.87 ± 1.82 g in excess twice a day for 10 days in two separate feeding trays allocated in 50 tanks of 0.5 m^3. Survival reached 93.3 ± 5.80% and was unaffected by supplementation. Final BW was the highest for shrimp fed the KRM-supplemented diet (11.97 ± 0.93 g), followed by POS (11.11 ± 0.77 g) and SQM (11.01 ± 1.17 g). Diets SHM, SM, SLM, and NEG showed a lower shrimp BW than POS, but were not statistically different among them. Shrimp fed the SAH diet achieved the lowest BW (10.06 ± 1.02 g). The highest gained yield was obtained with diets KRM and

POS. No statistical difference was observed in shrimp yield among other diets. The lowest feed conversion ratio (FCR) was achieved with shrimp fed KRM (1.31 ± 0.05) when compared to diets SHM (1.47 ± 0.05), SAH (1.47 ± 0.07), and SLM (1.45 ± 0.17). Two-by-two comparisons indicated that shrimp preferred SHM and KRM, except when these were compared to SQM and SLM. No difference in feed preference was found between diets with SQM and SLM. SAH was the least preferred raw material in all comparisons. Results indicated that KRM acts as a powerful feeding effector and growth enhancer in fishmeal-challenged diets for whiteleg shrimp. A dietary supplementation with 3% KRM is more effective than the same dose of any other chemoattractant evaluated.

KEYWORDS

chemoattractants, feed stimulation, growth enhancement, shrimp

1 | INTRODUCTION

In 2017, a total of 4.5 million m.t. of the whiteleg shrimp, *Litopenaeus vannamei*, were harvested from aquaculture farms, accounting for 81% of the global output of shrimp production alone (Cai, Zhou, Yan, & Lagana, 2019). Rapid animal growth is one of the most sought after parameters in shrimp farming. A high growth rate can improve production efficiency as it reduces farm operational costs and economic risks and increases annual yields. This is achieved through early harvests and a greater crop turnover. Protein is the major nutrient associated with tissue synthesis in animals. In shrimp feeds, protein quantity and quality are both required to obtain optimal digestion, assimilation, and thus growth. In recent years, there has been a strong movement toward low fishmeal shrimp feeds (Tacon & Metian, 2008), resulting in a greater dietary inclusion of plant and rendered animal by-products (Malcorps et al., 2019; Suresh, Paramasivam, Vasagam, & Nates, 2011). Such changes can severely impact shrimp growth, as these alternate proteins are often deficient in one or more essential nutrients (Nunes, Sá, Browdy, & Vázquez-Añón, 2014; Sá, Sabry-Neto, Cordeiro-Júnior, & Nunes, 2013) and contain antinutritional factors, which can suppress feeding stimulus and reduce nutrient bioavailability (Gatlin et al., 2007; Nunes, Sá, Andriola-Neto, & Lemos, 2006; Sabry-Neto, Lemos, Raggi, & Nunes, 2017).

Practical shrimp feed formulations have traditionally relied on meals, solubles, and hydrolysates made from fish, squid, shrimp, krill, and mollusks to act as chemoattractants and feeding stimulants (Cruz-Ricque, Guillaume, Cuzon, & Aquacop, 1987; Cruz-Suárez, Guillaume & Wormhoudt, 1987; Guillaume, Cruz-Ricque, Cuzon, Wordmhoudt, & Revol, 1989; Lee & Meyers, 1997; Smith, Tabrett, Barclay, & Irvin, 2005; Grey, Forster, & Dominy, 2009; Nunes et al., 2006; Suresh et al. 2011; Derby et al., 2016). These raw materials contain natural chemical drivers, which activate shrimp feeding behavior by promoting feed detection, and search and orientation toward the food source (known as chemoattractants). Some can also stimulate feeding activity through initiation and continuation of feeding (Costero & Meyers, 1993; Derby et al., 2016; Lee & Meyers, 1996, 1997; Nunes et al., 2006). These positive behavioral feeding responses appear to ultimately lead to a growth-enhancement effect in penaeid shrimp (Cruz-Ricque et al., 1987; Cruz-Suárez et al., 1987; Guillaume et al., 1989; Córdova-Murueta & García-Carrenõ, 2002; Smith et al.,

2005; Williams, Smith, Barclay, Tabrett, & Riding, 2005; Suresh et al. 2011). In the tiger shrimp, *Penaeus monodon*, Williams et al. (2005) demonstrated that the insoluble protein constituent of crustacean-based feed ingredients has the ability to enhance growth. The authors found that shrimp growth increased curvilinearly from 0.95% per day in a basal diet to 1.66% and 1.68% in diets that contained 15% shrimp head meal (SHM) and 15% whole- dried krill meal (KRM). Similarly, Smith et al. (2005) reported that juvenile tiger shrimp grew about 20% faster on feeds containing crustacean meal or KRM. In the whiteleg shrimp, *L. vannamei*, Córdova-Murueta and García-Carreño (2002) found that a krill hydrolysate enhanced shrimp growth, when it replaced 3, 5, and 15% of the total dietary crude protein (CP) of a commercial feed.

Marine chemoattractants have also been able to offset the effects of fishmeal replacement when used at supplemental dietary levels between 1 and 3%. Suresh et al. (2011) raised the blue shrimp, *L. stylirostris*, with a fishmeal-deprived diet containing 20% poultry meal. The authors reported that feed attractability was improved with 3% squid liver meal (SLM) or KRM, but feed palatability and shrimp growth were only enhanced with KRM. In a soy-based diet with 5% fishmeal and 1% fish oil, Sá et al. (2013) found that a combination of KRM and squid meal (SQM) at 2% significantly enhanced whiteleg shrimp final body weight (BW). Sabry-Neto et al. (2017), working with all plant-protein diets for *L. vannamei*, were able to stimulate feed intake with only 1% KRM. At 2% dietary inclusion, KRM accelerated shrimp growth, increased yield, and reduced feed conversion ratio.

In a previous study, Nunes et al. (2006) evaluated nine commercial feed attractants and stimulants for juvenile *L. vannamei* using a Y-maze aquarium apparatus. The authors were able to discriminate their efficacy in terms of stimulatory feeding responses (i.e., time spent on feed detection, orientation, locomotion, and feeding activity). The present study aimed at comparing feed preference and the ability of selected marine chemoattractants to enhance the growth of juvenile *L. vannamei* fed a fishmeal-challenged diet.

2 | MATERIAL AND METHODS

2.1 | Raw materials and diets

Marine chemoattractants selected for this study were chosen based on previous studies, where they have shown their ability to elicit a positive feeding response in marine shrimp (*P. monodon*, Cruz-Suárez et al. 1987; Fox, Blow, Brown, & Watson, 1994; Aquacop and Cuzon, 1989; Smith et al., 2005; Williams et al., 2005; *L. vannamei*, Cruz-Suárez et al. 1987; Córdova-Murueta & García-Carreño, 2002; Nunes et al., 2006; *L. stylirostris*, Cruz-Suárez et al. 1987; Aquacop and Cuzon, 1989; Grey et al., 2009; Suresh et al. 2011; *Marsupenaeus japonicus*, Cruz & Guillaume, 1983; Cruz-Ricque et al., 1987; Cruz-Suárez et al. 1987; and *Fenneropenaeus indicus*, Cruz-Suárez et al. 1987). SQM, SHM, shrimp meal (SM), and SLM were provided as a courtesy of an Indian feed manufacturer. Sardine hydrolysate (SAH), salmon meal (POS), and soy protein concentrate (NEG) were purchased in Brazil directly from suppliers and KRM from Aker BioMarine Antarctic AS from Norway.

All chemoattractants (except SM) and finished diets were chemically analyzed (AOAC, 2005). SM contained an excessive amount of ash, which had to be removed by proper grinding, thus decreasing its availability for analysis. Dry matter (DM) was determined by drying samples in a convection oven for 24 hr at 105°C. The Dumas combustion method was applied to analyze CP (AOAC 968.06), while crude fat was determined through acid hydrolysis (AOAC 954.02). Ash content was determined by burning samples in a muffle furnace at 600°C for 2 hr (AOAC 942.05) and crude fiber by enzymatic-gravimetric determination (AOAC 992.16). Calcium and phosphorous were analyzed via atomic absorption spectrometry and alkalimetric ammonium molybdophosphate method (AOAC 964.06), respectively. Amino acid (AA) and fatty acid compositions were determined using high-performance liquid chromatography (Hagen, Frost, & Augusti, 1989; White, Hart, & Fry, 1986) and high-resolution gas chromatography (GC) with flame ionization detection fitted with a capillary GC column, respectively. The peroxide value of fat was analyzed via titration (AOAC 965.33). Biogenic amines were quantified by a rapid and selective cation exchange chromatographic method coupled to integrated pulsed amperometry detection in accordance with a study by Pastore et al. (2005).

Raw materials showed a DM content varying from 27.20 (SAH) to 91.63% (KRM, Table 1). SQM contained the highest level of CP (75.22%, as-is basis) in contrast to SAH being the lowest (17.62%). Lipid content was the highest in KRM (25.16%) and the lowest in SAH (2.26%). With regard to fatty acid profile (% of total fatty acids, on a DM basis), the highest percentage of highly unsaturated fatty acids (HUFA, on a DM basis) was observed in SAH (45.52% of total fatty acid), followed by KRM (26.23%). In comparison, SQM contained the lowest level of these fatty acids (Table 2).

Feed formulation consisted of designing a practical diet with 3.00% fishmeal, no chemoattractants, and a high dietary inclusion of plant ingredients. Soybean protein was used in excess of 50% (43.54% soybean meal and 9% NEG), while dietary inclusion of wheat flour and wheat gluten meal reached 29.39% (Table 3). The basal diet was supplemented with the following free AAs: DL-methionine, L-lysine, and L-threonine. From this practical diet, eight other diets were prepared, supplemented with 3% of each raw material. The exception was SAH, which was added at 5% (1.36% on a DM basis) to account for its diluted content, but not more than 5% in order to avoid an excessive increase in feed moisture content. All chemoattractants were included during mixing of feed ingredients, prior to pelleting. Diets were manufactured with laboratory equipment as described in Nunes, Sá, and Sabry-Neto (2011).

Dietary CP, lipid, fiber, and ash levels (% of the diet, as-is basis; mean ± *SD*) reached 35.7 ± 0.5, 7.8 ± 1.8, 3.6 ± 0.4, and 9. 6 ± 0.2%, respectively (Table 3). The essential amino acid (EAA) composition varied on average 5.4% (CV, coefficient of variation) between finished diets (Table 4). Total dietary lysine, methionine (Met), and Met + Cys (cysteine) reached 2.1 ± 0.09, 0.74 ± 0.04, and 1.17 ± 0.08% (% of the diet, as-is basis), respectively.

2.2 | Shrimp growth performance

Growth performance was evaluated in 56 outdoor circular tanks of 1 m^3 (bottom surface area of 1.02 m^2, 0.74 m height, and 1.14 m diameter), allowing seven replicate tanks for each experimental diet. Shrimp of 0.99 ± 0.08 g (n = 5,712) were stocked under 100 animals/m^2 and fed 10 times during daylight using an automatic feeding dispenser (Nunes, Sabry-Neto, Silva, Oliveira-Neto, & Masagounder, 2019). Shrimp were fed following a feeding table, according to the equation $MM = 0.0931BW^{0.6200}$ (Façanha, Sabry-Neto, Figueiredo-Silva, Oliveira-Neto, & Nunes, 2018; Nunes et al., 2006; Nunes & Parsons, 2000), where MM is the maximum amount of feed (g) that can be eaten daily by one individual of a specific BW. To avoid excess feeding, feeding rates were reduced by 20% across all diets. Feeding rates changed daily based on estimated shrimp weight gain and by applying a fixed daily drop in shrimp survival of 0.06%. Starting on Day 10, meals were corrected by weighing 10 shrimp in bulk per tank on a weekly basis. Feed remains, if any, were not collected throughout the culture period.

The rearing system was operated with a minimum water exchange and continuous aeration to reach near saturation of dissolved oxygen. Nitrogen compounds were controlled by weekly application of dried sugar cane molasses at 5 g/m^3. Freshwater was added whenever needed to compensate for water losses because of evaporation and to control the increase in water salinity. Water temperature, salinity, and pH remained stable during culture, at 30.0 ± 1.0°C (n = 3,077), 34 ± 2 g/L (n = 3,078), and 7.9 ± 0.2 (n = 3,079), respectively.

At harvest, shrimp growth performance was determined by counting and weighing animals in 0.01-g precision electronic scales. Shrimp final survival (S, %) was calculated by the equation: $S = (POP_f/POP_i) \times 100$, where POP_i = the number of shrimp at stocking, and POP_f = the number of shrimp at harvest. Weekly weight gain (WWG, g/week) was determined by the formula: $WWG = [(W_f - W_i) \div t] \times 7$, in which W_i = shrimp wet BW (g) at stocking, W_f = shrimp BW (g) at harvest, and t = the number of days in culture. Gained shrimp yield (YIE, g of shrimp biomass gained/m^2) was determined as YIE = (BIO_f – BIO_i)/tank bottom area (m^2), where BIO_i = initial shrimp biomass per tank (g), BIO_f = 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.

TABLE 1 Proximate, amino acid, and biogenic amine composition of attractants[a] used in this study

	Proximate/amino acid composition (%, as-is basis)							
Nutrient	**POS**	**NEG**	**KRM**	**SQM**	**SHM**	**SAH**	**SM**	**SLM**
Dry matter	89.11	94.65	91.63	89.00	90.23	27.20	na[b]	89.39
Crude protein	64.44	62.24	55.00	75.22	52.95	17.62	na	48.74
Crude lipid	8.71	2.35	25.16	4.06	6.43	2.26	na	6.02
Total fiber	0.21	3.31	3.06	1.31	14.37	0.08	na	5.68
Ash	16.12	6.80	8.55	14.89	21.81	7.83	na	7.86
Calcium	3.33	0.35	1.25	4.36	5.75	0.53	na	0.51
Phosphorous	2.52	0.75	1.23	1.14	1.39	1.81	na	0.56
Essential amino acids[c] (EAA)								
Arginine	3.91	4.56	3.34	4.56	3.55	0.93	na	3.29
Histidine	1.77	1.59	1.31	2.73	1.43	0.58	na	1.24
Isoleucine	2.67	2.90	2.73	4.27	2.39	0.69	na	2.31
Leucine	4.36	4.79	4.22	5.96	3.90	1.14	na	3.49
Lysine	4.97	3.76	3.86	6.24	3.57	1.41	na	3.28
Methionine	1.87	0.80	1.54	2.18	1.29	0.46	na	0.67
Met + Cys[d]	2.70	1.62	1.95	3.04	1.81	0.88	na	1.27
Phenylalanine	2.51	3.27	2.90	3.51	2.14	0.67	na	2.49
Threonine	2.76	2.45	2.38	3.28	2.31	0.62	na	1.83
Tyrosine	1.91	2.25	3.25	3.12	1.78	0.50	na	1.70
Valine	3.06	2.97	2.83	3.90	2.66	0.79	na	2.16
Non-essential amino acids (NEAA)								
Alanine	4.25	2.68	2.99	4.08	3.45	1.07	na	2.18
Aspartic acid	4.82	7.04	5.73	8.45	5.44	1.72	na	6.26
Cysteine	0.83	0.82	0.41	0.86	0.52	0.42	na	0.60
Glycine	5.75	2.61	2.58	3.39	4.32	1.10	na	2.34
Glutamic acid	7.37	11.18	7.05	9.17	7.11	2.31	na	8.08
Proline	3.34	3.18	2.18	2.93	3.05	0.69	na	2.43
Serine	2.95	3.18	2.22	3.13	2.41	0.66	na	2.47
Taurine	0.91	—[e]	0.14	0.57	0.47	0.18	na	0.11
Sum EAA	29.79	29.34	28.37	39.75	25.02	7.79	na	22.46
Sum NEAA	30.22	30.69	23.29	32.58	26.77	8.15	na	24.47
EAA + NEAA	60.01	60.03	51.66	72.33	51.79	15.94	na	46.93
Biogenic amines (mg/kg)								
Cadaverine	na	na	—	na	90.80	—	na	33.30
Putrescine	na	na	—	na	271.87	—	na	48.19
Tyramine	na	na	—	na	59.80	—	na	32.69
Histamine	na	na	—	na	—	—	na	—

[a]POS, salmon meal; NEG, soy protein concentrate; KRM, krill meal; SQM, squid meal; SHM, shrimp head meal; SAH, sardine hydrolysate; SM, shrimp meal; SLM, squid liver meal.
[b]Not available.
[c]All EAA reported, except tryptophan.
[d]TSAA, total sulfur amino acids.
[e]—, not detectable.

TABLE 2 Fatty acid profile of attractants used in this study

		Fatty acid profile (% of total dietary fatty acid, dry matter basis)						
Fatty acid	POS	NEG	KRM	SQM	SHM	SAH	SM	SLM
12:0	0.11	na[a]	0.24	0.25	0.02	0.04	na	0.17
14:0	2.87	na	14.19	4.43	1.56	6.64	na	6.48
15:0	0.23	na	0.48	1.48	0.78	1.77	na	0.83
16:0	18.14	na	26.75	38.67	23.79	26.99	na	25.25
17:0	0.34	na	0.12	2.96	1.56	2.21	na	1.16
18:0	4.94	na	1.35	16.01	8.09	7.52	na	6.31
14:1	0.11	na	0.48	0.25	0.31	0.44	na	0.17
15:1	0.01	na	0.08	0.25	0.16	0.04	na	0.17
16:1n-7	3.67	na	9.66	5.91	3.27	3.54	na	6.15
18:1n-9*t*	0.57	na	0.87	0.49	0.16	0.04	na	0.02
18:1n-9	27.90	na	14.11	18.23	17.88	0.04	na	11.30
20:1n-9	1.84	na	0.99	—	—	0.44	na	0.50
22:1n-9	—	na	0.00	—	—	0.44	na	—
24:1n-9	—	na	—	—	0.78	0.44	na	—
18:2n-6*t*	—	na	0.24	—	—	0.04	na	—
18:2n-6	12.97	na	1.99	1.48	19.60	2.21	na	12.62
20:2n-6	0.80	na	0.08	0.49	2.02	0.44	na	0.33
18:3n-6	0.23	na	0.28	—	—	0.04	na	0.33
20:3n-6	0.57	na	0.12	0.25	0.31	0.44	na	0.33
18:3n-3	3.67	na	1.59	0.25	1.56	1.33	na	1.83
20:3n-3	0.34	na	0.12	—	0.47	0.04	na	0.17
20:4n-6	1.38	na	0.32	0.25	3.27	2.65	na	2.49
20:5n-3	7.69	na	17.17	2.22	7.47	8.41	na	10.30
22:2n-6	0.01	na	—	0.25	—	0.04	na	0.17
22:6n-3	11.37	na	8.74	3.94	7.47	31.42	na	12.79
n-3[b]	23.08	na	27.62	6.40	16.95	41.19	na	25.08
n-6[c]	15.97	na	3.02	2.71	25.19	5.88	na	16.28
n-9[d]	30.31	na	15.98	18.72	18.82	1.42	na	11.81
SFAs[e]	26.64	na	43.12	63.79	35.79	45.18	na	40.20
MUFAs[f]	34.11	na	26.19	25.12	22.55	5.44	na	18.44
PUFAs[g]	18.60	na	4.41	2.46	23.95	4.56	na	15.61
HUFAs[h]	20.45	na	26.23	6.65	18.20	42.52	na	25.75
EFAs[i]	35.71	na	29.49	7.88	36.08	43.36	na	37.54

[a]Not analyzed.
[b]n-3, 18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3.
[c]n-6, 18:2n-6*t*, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, and 22:2n-6.
[d]n-9, 18:1n-9*t*, 18:1n-9, 20:1n-9, 22:1n-9, and 24:1n-9.
[e]SFAs, saturated fatty acids, 12:0, 14:0, 15:0, 16:0, 17:0, and 18:0.
[f]MUFAs, monounsaturated fatty acids, 14:1, 15:1, 16:1, 18:1, 20:1, 22:1, and 24:1.
[g]PUFAs, polyunsaturated fatty acids, 18:2, 18:3, 20:2, and 20:3.
[h]HUFAs, highly unsaturated fatty acids, 20:4, 20:5, 22:2, and 22:6.
[i]EFAs, essential fatty acids, 18:2n-6, 18:3n-3, 20:5n-3, and 22:6n-3.

TABLE 3 Ingredient and proximate composition of experimental diets

Ingredient	Ingredient composition (% of the diet, as-is basis)							
	POS	NEG	KRM	SQM	SHM	SAH	SM	SLM
Soybean meal[a]	43.54	43.54	43.54	43.54	43.54	43.54	43.54	43.54
Wheat flour	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soy protein concentrate[b]	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Vital wheat gluten[c]	4.39	4.39	4.39	4.39	4.39	4.39	4.39	4.39
Soy lecithin	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36
Salmon meal[d]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salmon oil	2.66	2.66	2.66	2.66	2.66	2.66	2.66	2.66
Calcium carbonate	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59
MSP[e]	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Salt	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Magnesium sulfate	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19
Potassium chloride	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Vitamin–mineral premix[f]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Synthetic binder[g]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine[h]	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
DL-methionine[i]	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Cholesterol[j]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Ascorbic acid[k]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-threonine[l]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Dietary supplementation (%, as is)								
Salmon meal	3.00	–	–	–	–	–	–	–
SPC	–	3.00	–	–	–	–	–	–
Krill meal[m]	–	–	3.00	–	–	–	–	–
Squid meal	–	–	–	3.00	–	–	–	–
Shrimp head meal	–	–	–	–	3.00	–	–	–
Sardine hydrolysate	–	–	–	–	–	5.00	–	–
Shrimp meal[n]	–	–	–	–	–	–	3.00	–
Squid liver meal	–	–	–	–	–	–	–	3.00
Proximate composition (% of the diet, as is basis)								
Dry matter	89.12	na[o]	89.98	90.37	90.06	88.01	90.75	89.34
Crude protein	36.08	na	35.99	36.36	35.55	35.04	35.90	35.15
Crude fat	7.34	na	7.27	7.26	7.19	6.99	7.12	7.13
Crude fiber	3.41	na	3.40	3.56	4.21	3.09	3.63	3.78
Crude ash	9.69	na	9.56	9.56	9.62	9.50	9.88	9.09
Calcium	1.36	na	1.30	1.43	1.42	1.30	1.33	1.24
Phosphorous	0.88	na	0.85	0.86	0.86	0.92	0.86	0.84

(Continues)

TABLE 3 (Continued)

Ingredient	Ingredient composition (% of the diet, as-is basis)							
	POS	NEG	KRM	SQM	SHM	SAH	SM	SLM
NFE[p]	32.60	na	33.76	33.63	33.49	28.39	34.22	34.19
GE (MJ/kg)[q]	19.31	na	19.33	19.36	19.28	20.22	19.26	19.32

[a]Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil).
[b]Sementes Selecta S.A. (Araguari, Goiás, Brazil).
[c]Amytex 100. Tereos Syral S.A.S. (Marckolsheim, France).
[d]Pesquera Pacific-Star (Puerto Montt, Chile).
[e]Monosodium phosphate.
[f]Vaccinar Industria e Comercio Ltda. (Pinhais, Brazil). Guarantee levels per kg of product: vitamin A, 1,200,000 IU; vitamin D_3, 200,000 IU; vitamin E, 60,000 mg; vitamin K_3, 1,000 mg; vitamin B_1, 2,400 mg; vitamin B_2, 2,400 mg; vitamin B_6, 6,000 mg; vitamin B_{12}, 4 mg; nicotinic acid, 10,000 mg; pantothenic acid, 5,200 mg; biotin, 20 mg; folic acid, 400 mg; vitamin C, 30,000 mg; choline, 50,000 mg; inositol, 80,000 mg; Fe 26,000 mg; Cu, 2,000 mg; Zn, 20,000 mg; Mn, 5,000 mg; Se, 100 mg; I, 600 mg; Co, 105 mg; and Cr, 60 mg.
[g]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%).
[h]Biolys®, L-lysine 54.6%. Evonik Nutrition and Care GmbH (Hanau, Germany).
[i]DL-methionine 99%. Evonik Nutrition and Care GmbH (Hanau, Germany).
[j]Cholesterol SF, minimum of 91% of active cholesterol; Dishman Netherlands B.V. (Veenendaal, Netherlands).
[k]Rovimix® Stay C® 35. Minimum of 35% of phosphorylated vitamin C activity. DSM Nutritional Products AG (Schweiz, Switzerland).
[l]ThreAMINO®, L-threonine, Feed Grade 98.5%. Evonik Nutrition and Care GmbH (Hanau, Germany).
[m]Qrill™ Antarctic krill meal (full fat), Aker Biomarine Antarctic AS (Lysaker, Norway).
[n]Sun-dried *Acetes* spp.
[o]na, not available.
[p]Nitrogen-free extract. Calculated by difference [dry matter – (crude protein + fat + crude fiber + ash)].
[q]Gross energy (GE, MJ/kg) given on a DM basis. Calculated as GE = (4,143 + (56 × lipid [% DM]) + (15 × crude protein [% DM]) – (44 × crude ash [% DM])) × 0.0041868. Source: Ewan (1989).

2.3 | Feed preference

Feed preference was assessed following the method described in Browdy, Bharadwaj, Venero, and Nunes (2012) with minor modifications. The method consists of simultaneously confronting two similar diets supplemented with different chemoattractants and measuring their relative AFI values. The relative AFI (%) value was calculated by dividing the amount of dried uneaten feed (g) recovered from feeding trays after 1 hr of water exposure by the amount of dried feed offered (g) multiplied by 100.

Shrimp of 10.87 ± 1.82 g (n = 2,000) were stocked in 50 indoor tanks of 0.5 m^3 at 70 animals/m^2 (40 shrimp per tank). Tanks operated with no water exchange and were equipped with two round feeding trays (141 mm^2 in area each) resting on the tank bottom placed opposite to each other. Shrimp were fed in excess exclusively in feeding trays twice a day, at 07:30 a.m. and 13:30 p.m. A 3-day acclimation period with a commercial grower shrimp feed was allowed before starting a 10-day assessment period with the experimental diets. After each feeding time, feed leftovers were recovered from feeding trays and dried in a convection oven at 105°C for 24 hr to calculate AFI.

For validation purposes, the positive (POS) and negative (NEG) control diets were compared against each other and against individual diets supplemented with chemoattractants KRM, SQM, SHM, SM, SLM, and SAH. Another set of 16 two-by-two comparisons with selected chemoattractants was carried out: KRM versus SM, SAH, SHM, SLM, and SQM; SM versus SAH, SHM, SLM, and SQM; SLM versus SM, SAH, SHM, and SQM; SQM versus SHM, and SAH; and SAH versus SHM. Three tanks were assigned for each set of comparisons, except POS versus NEG, which used four replicate tanks.

TABLE 4 Amino acid composition of experimental diets[a]

	Proximate/amino acid composition (%, as-is basis)							
Nutrient	**POS**	**NEG**	**KRM**	**SQM**	**SHM**	**SAH**	**SM**	**SLM**
Essential amino acids (EAA)								
Arginine	2.29	na[b]	2.27	2.33	2.25	2.21	2.03	2.11
Histidine	1.00	na	1.05	0.97	0.98	0.99	0.89	0.94
Isoleucine	1.71	na	1.64	1.70	1.69	1.61	1.47	1.55
Leucine	2.67	na	2.59	2.67	2.58	2.59	2.41	2.49
Lysine	2.22	na	2.13	2.18	2.15	2.12	1.96	2.01
Methionine	0.77	na	0.79	0.68	0.71	0.75	0.73	0.73
Met + Cys[c]	1.25	na	1.15	1.28	1.16	1.19	1.03	1.13
Phenylalanine	1.84	na	1.67	1.84	1.81	1.80	1.63	1.71
Threonine	1.34	na	1.30	1.31	1.27	1.28	1.14	1.19
Tyrosine	1.23	na	1.20	1.23	1.20	1.18	1.05	1.11
Valine	1.69	na	1.63	1.72	1.66	1.56	1.44	1.53
Non-essential amino acids (NEAA)								
Alanine	1.61	na	1.58	1.6	1.56	1.55	1.43	1.45
Aspartic acid	0.48	na	0.36	0.6	0.45	0.44	0.3	0.40
Cysteine	1.66	na	1.57	1.64	1.58	1.55	1.43	1.46
Glycine	1.77	na	1.79	1.78	1.7	1.77	1.6	1.66
Glutamic acid	2.25	na	2.22	2.24	2.19	2.18	1.99	2.06
Proline	3.44	na	3.78	3.66	3.59	3.62	3.29	3.48
Serine	7.18	na	7.14	7.1	7.08	7.07	6.42	6.71
Taurine	0.06	na	0.05	0.05	0.06	0.05	0.07	0.04
Sum EAA	16.76	na	16.27	16.63	16.30	16.09	14.75	15.37
Sum NEAA	18.45	na	18.49	18.67	18.21	18.23	16.53	17.26
EAA + NEAA	35.21	na	34.76	35.30	34.51	34.32	31.28	32.63

[a]Each diet contained 3% POS (salmon meal), 3% NEG (soy protein concentrate), 3% KRM (krill meal), 3% SQM (squid meal), 3% SHM (shrimp head meal), 5% SAH (sardine hydrolysate), 3% SM (shrimp meal), or 3% SLM (squid liver meal).
[b]na, not available.
[c]TSAA, total sulfur amino acids.

2.4 | Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences, package 23 (IBM® SPSS® Statistics, Chicago, Illinois). The effect of dietary supplementation of each chemoattractant on shrimp growth performance was analyzed through one-way ANOVA. When significant differences were detected, two-by-two comparisons were carried out using the Tukey's honestly significant difference (HSD) test. Student's *t* test was applied to compare the differences in AFI when diets were compared against each other over the feed preference assay (Figure 2).

3 | RESULTS

At harvest, shrimp survival was high, reaching a mean of 93.3 ± 5.80%. Survival was unaffected by the dietary supplementation of raw materials ($p > .05$, Table 5). However, shrimp final BW, weekly growth, gained yield, AFI, and

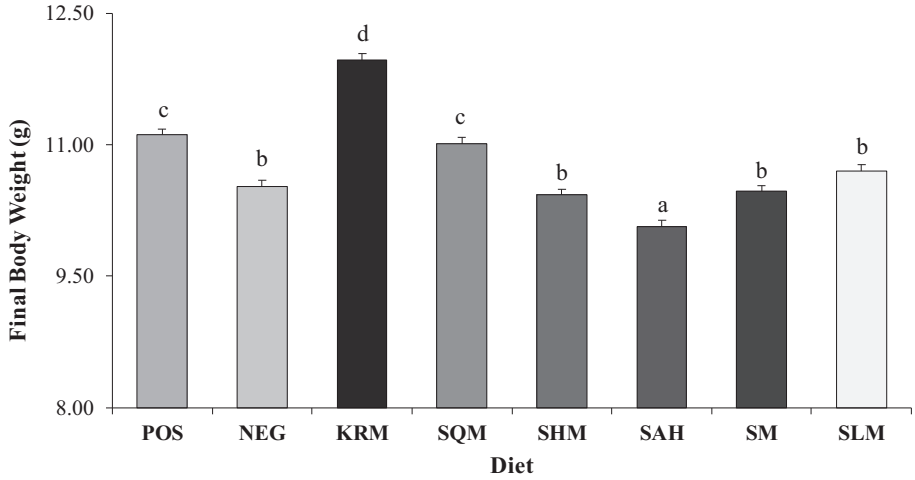

FIGURE 1 Mean (±*SE*) body weight (g) of *Litopenaeus vannamei* after 74 days of rearing in green water tanks of 1 m^3. Different letters indicate statistically significant differences between diets at the α = 0.05 level by Tukey's honestly significant difference (HSD). POS, salmon meal; NEG, soy protein concentrate; KRM, krill meal; SQM, squid meal; SHM, shrimp head meal; SAH, sardine hydrolysate; SM, shrimp meal; SLM, squid liver meal

FCR were significantly different within dietary treatments ($p < .05$). Final BW was statistically higher for shrimp fed diets supplemented with KRM (11.97 ± 0.93 g, Figure 1). This was followed by shrimp fed the POS (11.11 ± 0.77 g) and SQM (11.01 ± 1.17 g) diets. Shrimp fed SAH achieved the lowest BW (10.06 ± 1.02 g) among experimental diets even when compared with the NEG control (10.52 ± 0.58 g). Shrimp fed diets SHM, SM, SLM, and NEG showed a lower BW than those fed POS, but were not statistically different among each other ($p > .05$). Shrimp grew significantly faster when fed KRM compared to SAH, but did not differ when compared to other diets. The highest gained yield was obtained when shrimp were fed the diets KRM and POS. No statistical difference was observed in shrimp yield among other dietary treatments ($p > .05$). In comparison to KRM, diets SHM, SAH, and NEG showed a lower response in AFI. The lowest FCR was achieved with shrimp fed KRM when compared to diets SHM, SAH, and SLM.

Validation of the feed preference assay showed consistent responses when the NEG control diet was compared with diets supplemented with the different chemoattractants. There was a statistically lower AFI (%) for the NEG control when compared with diets supplemented with marine chemoattractants, including POS ($p < .05$). The only exception was observed when two-by-two comparisons were carried out between NEG and diets with 3% SLM and 5% SAH ($p > .05$). The diet containing SLM proved to be less attractive compared to POS ($p < .05$). However, when POS was compared with SAH, shrimp preferred the former ($p < .05$). AFI was also significantly higher for diets KRM, SM, and SQM in comparison to POS. No difference could be observed when POS was compared to SHM.

When comparisons were made between different chemoattractants, AFI above 80% was only detected when KRM was compared to SM and SAH. In both cases, AFI was statistically higher for KRM ($p < .05$). No difference in AFI could be observed when KRM or SHM were compared to SQM and SLM ($p < .05$). However, a significantly higher AFI was found for SHM compared to KRM, SM, and SAH. Relative AFI for SQM and SLM did not differ statistically between each other, and only the former was higher than SM ($p < .05$). SAH showed the lowest feed preference score, except when compared with SLM ($p < .05$). SAH performed similar to SLM, but inferior to KRM, SM, SHM, and SQM.

The analysis of biogenic amines in selected chemoattractants showed undetectable levels of cadaverine, putrescine, tyramine, and histamine in both KRM and SAH (Table 1). However, SHM and SLM achieved 90.80 and 33.30 mg/kg for cadaverine, 271.87 and 48.19 mg/kg for putrescine, and 59.80 and 32.69 mg/kg for tyramine. Histamine could not be detected in SHM and SLM.

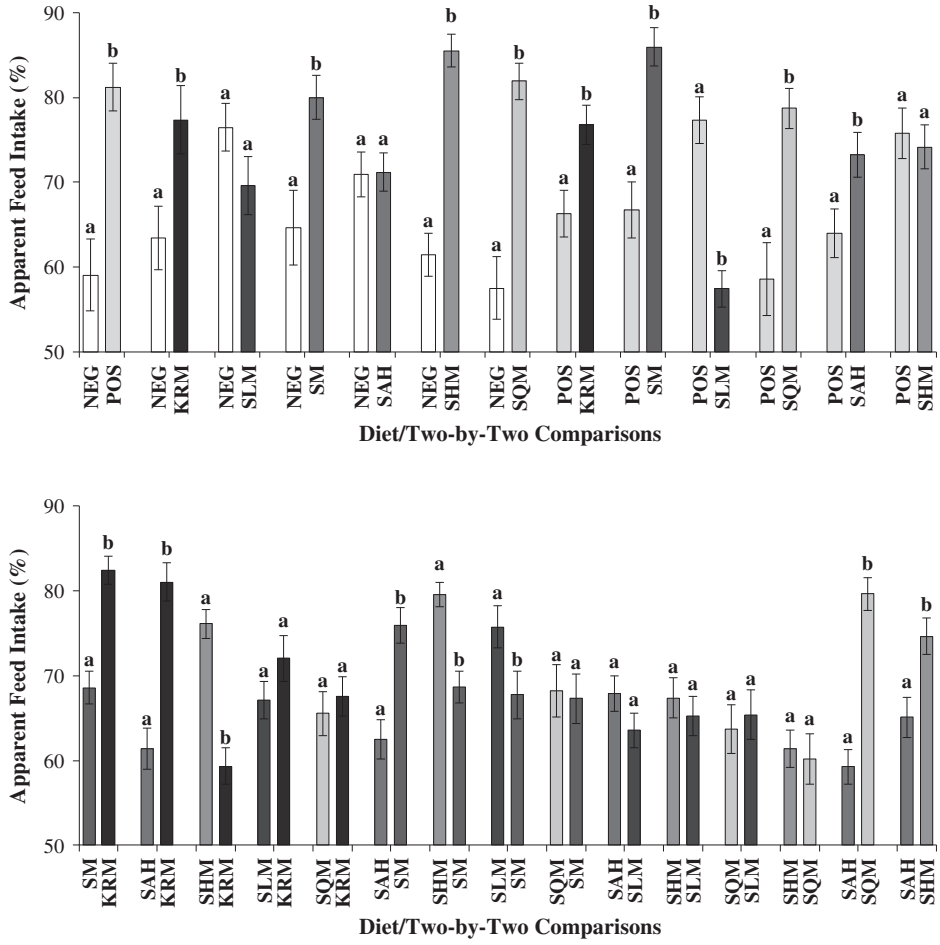

FIGURE 2 Two-by-two comparison of relative apparent feed intake (AFI, %) for juvenile *Litopenaeus vannamei* fed diets with different chemoattractants. Diets were confronted against each other over a 10-day period using two feeding trays per tank. Each bar represents the mean (± standard error) of 60 observations, except NEG vs. POS (n = 80). Different letters indicate statistically significant differences in AFI between diets at the α = 0.05 level in accordance to the student t-test. KRM, krill meal; SQM, squid meal; POS, salmon meal; SHM, shrimp head meal; SAH, sardine hydrolysate; SM, shrimp meal; SLM, squid liver meal; NEG, soy protein concentrate

4 | DISCUSSION

The results have demonstrated that not all marine chemoattractants examined have the ability to promote feed intake and growth of juvenile *L. vannamei* when used at supplemental dietary levels (between 3 and 5%, as fed basis) in a low fishmeal diet. The diet with 3% KRM remained the most effective in the increase of shrimp final BW and yield and in the reduction of FCR in *L. vannamei*. The ability of KRM in stimulating feed ingestion and growth performance in penaeid shrimp is corroborated by other studies conducted with both *P. monodon* and *L. vannamei* (Sá et al., 2013; Sabry-Neto et al., 2017; Smith et al., 2005; Williams et al., 2005). However, there was scarce information with regard to its performance relative to other marine raw materials with stimulatory feeding properties.

Suresh et al. (2011) compared the growth performance of 1.5 g *L. stylirostris* fed for 42 days using 21 self-cleaning microcosm tanks of 1,827 L. The authors formulated diets containing 20% pet-food grade poultry meal with

TABLE 5 Growth performance (mean ± *SD*) of *Litopenaeus vannamei* after 74 days of rearing

Diet[a]	Survival (%)	Growth (g/week)	Gained yield (g/m^2)	AFI[b] (g/shrimp)	FCR[c]
POS	92.4 ± 7.24	0.96 ± 0.07a,b	928 ± 40b	13.2 ± 0.4a,b	1.42 ± 0.05a,b
NEG	93.6 ± 5.47	0.89 ± 0.05a,b	872 ± 29a	12.6 ± 0.3a	1.44 ± 0.07a,b
KRM	94.8 ± 5.68	1.04 ± 0.09b	1,037 ± 73b	13.6 ± 0.6b	1.31 ± 0.05a
SQM	91.7 ± 8.84	0.96 ± 0.11a,b	911 ± 53a	12.9 ± 0.4a,b	1.41 ± 0.07a,b
SHM	91.6 ± 6.77	0.90 ± 0.10a,b	857 ± 44a	12.6 ± 0.6a	1.47 ± 0.05b
SAH	95.1 ± 2.77	0.86 ± 0.10a	858 ± 90a	12.5 ± 0.7a	1.47 ± 0.07b
SM	95.2 ± 4.44	0.90 ± 0.07a,b	899 ± 40a	12.7 ± 0.4a,b	1.41 ± 0.04a,b
SLM	92.0 ± 4.68	0.92 ± 0.13a,b	886 ± 119a	12.7 ± 0.7a,b	1.45 ± 0.17b
ANOVA *P*	0.848	0.026	< 0.0001	0.009	0.021

Note: Values in each column sharing different lowercase letters indicate significant differences ($p < .05$). When statistically significant, two-by-two comparisons were determined with the Tukey's HSD test.
[a]Each diet contained 3% salmon meal (POS), 3% soy protein concentrate (NEG), 3% KRM (krill meal), 3% SQM (squid meal), 3% SHM (shrimp head meal), 3% SM (shrimp meal), 3% SLM (squid liver meal), or 5% SAH (sardine hydrolysate).
[b]Apparent feed intake (g) is the amount of feed delivered divided by the number of stocked shrimp.
[c]Feed conversion ratio.

3% of anchovy fishmeal, SLM, fish hydrolysate, spray-dried blood meal, or KRM. Their results indicated that all ingredients included made no difference to shrimp growth, with the exception of KRM. Although SLM acted as an effective attractant and palatability enhancer at 3%, it did not promote growth. On the other hand, 3% KRM acted as an effective attractant, a palatability enhancer, and a growth promoter.

Data from our work corroborate the findings of Suresh et al. (2011). We have observed that juvenile *L. vannamei* feed preference is the same when diets with 3% SLM and 3% KRM were compared with each other. However, only the use of KRM resulted in a higher shrimp BW. In our study, shrimp fed SLM performed the same as the NEG control diet. This, in addition to the fact that POS was more attractive than SLM, indicated there was little value in supplementing 3% SLM. In comparison, shrimp fed KRM achieved a final BW and yield 13.7 and 18.9% higher than the NEG control, respectively, while FCR was reduced by 9.3%. Shrimp also fed more on a diet supplemented with 3% KRM than with POS. It is important to note that the chemical profile of our SLM was different from the one used by Suresh et al. (2011)as it contained lower levels of CP and EAA.

An unsettled debate remains regarding the growth-enhancing effect of certain marine chemoattractants in shrimp diets. There is speculation that shrimp growth could be promoted by their supplementary dietary nutritional values from their increased feeding stimuli and (or) due to unknown growth factors. Williams et al. (2005) working with *P. monodon* argued that the insoluble protein constituent of crustacean meals contained an unidentified growth factor. They carried out a series of experiments and rejected the possibility of a parallel improvement in the overall nutritional specification of their diets and, in particular, of an increased supply of some nutrients. In contrast, Cruz-Suárez et al. (1987) worked with a protein extracted from frozen squid (squid protein fraction, SPF). They reported that the growth rates of *L. vannamei*, *L. stylirostris*, and *F. indicus* were significantly enhanced when dietary supplementation of SPF was only 1.5%, while 6 and 16% were required for growth improvement in *P. monodon*. The authors explained that the growth enhancement effect at 16% was likely due to the improved dietary AA content, but related the low dietary inclusion effect to unknown growth factors. Guillaume et al. (1989) using SQM or extracts at 1.5, 3.0, 6.0, and 15% in a casein-based semi-purified diet found a monthly growth-enhancing effect of 30 to 50% in the Kuruma shrimp, *M. japonicus*. Their study indicated that shrimp fed SQM-supplemented diets increased cell hypertrophy, and the postprandial level of glucose and AA in hemolymph. However, they refused the hypothesis of an associated effect on shrimp growth with an improved feed digestion when SQM was used.

In our study, although chemoattractants evaluated contained marked differences in their nutrient composition, their inclusion resulted in nearly the same levels of CP, AA, and crude fat in experimental diets. Therefore, in agreement with previous studies, there is no clear indication that enhanced growth could have been driven by an increased dietary supply of these nutrients. Dietary EAA composition in our study, including Met + Cys, likely met minimum levels to optimize the growth of whiteleg shrimp under green-water rearing systems (Façanha, Oliveira-Neto, Figueiredo-Silva, & Nunes, 2016; Nunes, Sabry-Neto, & Masagounder, 2019). On the other hand, restricting fishmeal inclusion to 3% with only 2.66% fish oil in the NEG control diet may have impacted the supply of long-chain polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. Fatty acid profiles in raw materials KRM, POS, SAH, and SLM contained the highest concentrations of EPA and DHA. Thus, diets supplemented with 3% of KRM or POS may have provided additional levels of these LC-PUFAs improving shrimp performance. Comparatively, the high moisture content in SAH reduced any potential effect on the dietary levels of these fatty acids. Poor lipid freshness in SLM may have been detrimental to shrimp leading to its lower performance. Based on this, we support the premise that growth enhancement in low fishmeal diets is driven by ingredient freshness, a collective and stimulatory effect on feed intake, and a higher supply of key nutrients, possibly LC-PUFAs. This is corroborated by previous work. Sá et al. (2013) have shown that successful dietary reduction of fishmeal for juvenile *L. vannamei* is dependent on an adequate supply of LC-PUFAs. The authors have found that in a diet with 12% fishmeal, almost one-third can be substituted for SPC when 2% fish oil is adopted compared to no replacement at 1%. Further restriction of fishmeal to 5% with 1% fish oil was only successfully carried out when a 1:1 combination of KRM and SQM was used. Shrimp BW was significantly increased when incorporation of both chemoattractants summed up to 2% of the diet. Likewise, the authors speculated that enhancement of shrimp BW was probably driven by a positive effect on feed intake and a higher nutrient supply.

Our findings do not support the view that feed attractability alone improves the BW and FCR of *L. vannamei*. When SHM was compared against KRM, shrimp showed a higher preference for the former. However, the final BW in shrimp fed the SHM-supplemented diet was not enhanced beyond the NEG control or when compared to KRM. The fact that cadaverine, putrescine, and tyramine were all detected in SHM and SLM may have resulted in a higher feed intake, but compromised their nutrient quality. Biogenic amines are among the low molecular weight compounds known to act as feeding attractants and stimulants for shrimp (Lee & Meyers, 1997). However, observed concentrations were marginal (from 33 to 372 mg/kg in the analyzed raw material) compared to the thresholds evaluated to elicit positive feeding responses in shrimp. Mendoza, Montemayor, and Verde (1997) assessed the attractability efficacy of two biogenic amines (putrescine and cadaverine) with the freshwater prawn, *Macrobrachium rosenbergii*. They found that cadaverine at 2,000 mg/kg in a diet with 5% fishmeal acts as a good attractant. Tapia-Salazar, Smith, Harris, Cruz-Suárez, and Ricque-Marie (2004) worked with the blue shrimp, *L. stylirostris*, of 50 to 108 mg initial BW. They evaluated the dietary supplementation of cadaverine (0, 500, 1,100, 2,300, 3,500, and 4,600 mg/kg) for 28 days in 10-L fiberglass tanks. The authors found that dietary cadaverine supplementation has no nutritional value for shrimp and also has no effect on feed intake and shrimp growth.

SAH performed poorly in our study in terms of both feed preference and growth performance. This chemoattractant was made up of 72.80% water. In order to avoid final feed moisture levels in excess of 12%, dietary inclusion was limited to 5%. This inclusion is equivalent to 1.36% on a DM basis, almost half of the mean inclusion of 2.70 ± 0.03% (on a DM basis) adopted with other chemoattractants. Therefore, supplementation with SAH conferred little nutritional value to the finished feed, which may explain its poor performance as a growth enhancer. Also, a higher dietary inclusion of SAH may have been required to elicit a positive feeding response.

The literature presents little substantial evidence to support the use of fish hydrolysates as a feed attractant for shrimp. Smith et al. (2005) reported that a dried fish hydrolysate (9% moisture) included at 0.5, 1.0, and 2.0% in a feed with 3.6% fishmeal only showed a significant effect on daily feed intake of juvenile *P. monodon* when used at the highest inclusion level. However, after a 6-week rearing trial, they could not demonstrate a growth-enhancement benefit when fish hydrolysate was included at 2.0% into a feed with 17% fishmeal. Similarly, Suresh et al. (2011) working with a dried fish hydrolysate (6.8% moisture) included at 3% in a diet deprived of fishmeal reported that it

had little efficacy in attractability and palatability for juvenile *L. stylirostris*. The authors concluded that fish hydrolysate also performed relatively poorly in terms of growth enhancement after a 42-day rearing trial. Córdova-Murueta and García-Carreño (2002) replaced CP at 3, 9, and 15% in a commercial feed by liquid hydrolysates made from fish (mainly from Pacific whiting and bottom fish) and krill. After 54 days of rearing, the authors found that *L. vannamei* with an initial BW of 2.5 g grew better at all replacement levels when raised with feeds containing krill compared to fish hydrolysate. Grey et al. (2009) carried out a feeding stimulant bioassay using fish hydrolysates made from salmon by-products of the Alaska fish processing industry. Salmon hydrolysates were included at 5% into a reference diet containing 25% wheat flour and 75% soybean meal. The authors used a two-choice method to assess feed preference, similar to the one adopted in the present study. After a 4-day feeding period using *L. vannamei* of 5.8 ± 0.43 g, they concluded that salmon hydrolysates could serve as feeding stimulants. They noted that feed ingestion of diets containing hydrolysates was similar to a commercial shrimp feed. However, no controlled comparison was carried out between salmon hydrolysates and other chemoattractants.

In the present work, SM made from sun-dried *Acetes* spp. scored moderately in terms of feed preference, but low in its ability to promote shrimp growth. There was insufficient quantity of SM to carry out chemical analysis. However, SM probably contained lower levels of EAA than other chemoattractants as dietary EAA composition was slightly lower than the feed containing 3% SHM. This may have also contributed to the lower shrimp growth performance obtained with the SM-supplemented diet. The fact that SM was sun dried may have compromised its freshness and quality. Solar-dried shrimp by-products may contain higher peroxide values and lower levels of EAA and n-3 LC-PUFA, which can negatively affect shrimp growth performance (Fox et al., 1994). Previous studies have shown that increasing doses of SHM have a stimulatory effect on shrimp growth, but remain lower than KRM (Williams et al., 2005). The authors reported that the growth factor effect is more consistently present in Euphausid KRM than in other types of commercially available marine invertebrate protein sources. This is also in agreement with our findings.

5 | CONCLUSION

This work has shown that KRM acts as an effective feeding effector and growth enhancer in fishmeal-challenged diets for juvenile *L. vannamei*. A dietary supplementation with 3% KRM is more effective than the same dose of SQM, POS, SLM, SHM, and sun-dried SM or 5% liquid SAH. Other chemoattractants also had the ability to promote stimulatory effects on shrimp feed intake (e.g., POS and SQM), but without significant enhancement in BW and yield and reduction in FCR. Our findings support the view that the growth enhancement factor observed in KRM is apparently a positive balance between higher feed attractiveness and stimulation, and the supply of key dietary nutrients.

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ORCID

Alberto J. P. Nunes https://orcid.org/0000-0001-9105-8109

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