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Astaxanthin krill oil enhances the growth performance and fatty acid composition of the Pacific whiteleg shrimp, *Litopenaeus vannamei*, reared under hypersaline conditions

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Abstract

Hypersalinity culture of marine shrimp can lead to poor growth and feed efficiency. This study evaluated the effect of dietary supplementation of three oil sources (krill, fish and soybean) on the growth of *Litopenaeus vannamei* reared under high salinity. Shrimp of 2.79 ± 0.60 g were reared for 64 days under isosmotic (ISO, 23 ± 1.2 g/L) and hyperosmotic (HOS, 44 ± 2.0 g/L) conditions. Diets varied in their fatty acid composition: Control, 35 g/kg of the diet (as fed basis) soybean oil; Fish, 27 g/kg fish oil and 10 g/kg soybean oil; Krill, 48 g/kg krill oil and 4 g/kg soybean oil; Krill-, 15 g/kg krill oil and 21 g/kg soybean oil; Krill+, 55 g/kg krill oil and 4 g/kg soybean oil. At harvest, Krill diet promoted the fastest shrimp growth (1.01 ± 0.01 g/week) and body weight (11.97 ± 2.01 g), regardless of water salinity. There were no significant differences in shrimp survival (93.4 ± 5.07%) and yield (554 ± 68.5 g/m^2) among different diets. Shrimp fed Fish, Krill and Krill+ had higher concentrations of PUFA compared to those fed Control and Krill- diets.

KEYWORDS

essential fatty acids, fish oil, krill oil, *Litopenaeus vannamei*, water salinity

1 | INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, is known for its ability to withstand a wide spectrum of water salinities (Rodriguez, 1981). While the species isosmotic point has been determined at 718 mOsm (equivalent salinity of 24 g/L), *L. vannamei* can regulate efficiently both low and high salinities (Castille & Lawrence, 1981). Consequently, commercial farming of the white shrimp occurs in waters with less than 1 (Davis, Saoud, McGraw, & Rouse, 2002) to more than 50 g/L salinity (Perez-Velazquez et al., 2007).

Despite the ability to adapt to a wide range of salinity, hypo- and hyperosmoregulation lead to greater stress and energy intake often resulting in adverse effects to shrimp survival, growth and feed efficiency (Bray, Lawrence, & Leung-Trujillo, 1994; Ramos-Carreño et al., 2014; Sui, Ma, & Deng, 2015). In order to mitigate the effects of extreme salinity on shrimp culture, various studies have been conducted on dietary manipulation (carbohydrate, Wang et al., 2014; minerals, Gong, Jiang, Lightner, Collins, & Brock, 2004; Roy, Davis, Saoud, & Henry, 2007; Roy, Davis, Nguyen, & Saoud, 2009; Zhou, Zhang, Liu, & Ding, 2014; amino acids, Liu et al., 2016; crude protein, Sui et al., 2015; fatty acids, González-Félix, Perez-Velazquez, Quintero-Alvarez, & Davis, 2009; alternative protein sources, Roy, Bordinhon et al., 2009; cholesterol and lecithin, Gong et al., 2004; Roy, Davis, & Saoud, 2006) and pond management strategies (water ionic concentrations, Limhang, Limsuwan, Chuchird, & Taparhudee, 2011; feeding rates, Roy, Davis, & Whitis, 2012).

The dietary supplementation with long-chain polyunsaturated fatty acids (LC-PUFA), cholesterol, phospholipids and antioxidant nutrients has shown the most promising results in counteracting the negative effects of extreme salinities in *L. vannamei* culture (Gong et al., 2004; Hurtado et al., 2006; Liu, Wang, Wang, Wang, & Sun, 2007), although opposing evidence also exists (Hurtado et al., 2007; Roy et al., 2006). The role of these compounds in the osmoregulatory mechanisms is mainly supported by changes in cell membrane

composition, improved gill membrane permeability, antioxidant system support and increased energy contribution (Liu et al., 2007; Paula, Volkov, Van Hoek, Haines, & Deamer, 1996). Hurtado et al. (2006) observed a higher growth performance of juveniles of *L. vannamei* fed LC-PUFA-enriched diets for 21 days under 50 g/L salinity. The authors speculated that shrimp spent less energy on osmoregulation as a result of decreased membrane permeability in gills. Liu et al. (2007) fed *L. vannamei* for 35 days with diets supplemented with 0, 100 and 600 mg/kg of tocopheryl acetate. After a 24-hr acute salinity change, from 30 to 5 and 50 g/L, the authors reported a higher oxidative resistance in shrimp fed vitamin E-supplemented diets.

This study evaluated the effects of dietary supplementation of long-chain polyunsaturated fatty acids (LC-PUFA) on the growth performance, fatty acid composition and sensory characteristics of the tail of juvenile *L. vannamei* reared under high salinity. Shrimp were fed diets with three oil sources (krill, fish and soybean) under an isosmotic and hyperosmotic condition.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This study used a complete randomized design in a 2 × 3 factorial arrangement with two salinities and three diets with a minimum of six replicate tanks per dietary treatment. Water salinity was maintained between 20 and 26 g/L (minimum and maximum), corresponding to a near isosmotic (ISO)–osmotic range for *L. vannamei* (Castille & Lawrence, 1981; Li et al., 2008), and between 40 and 47 g/L, corresponding to a hyperosmotic (HOS) condition (Perez-Velazquez et al., 2007; Rodriguez, 1981).

Three diets were formulated to contain a nearly similar ingredient composition, except in regard to the inclusion of soybean oil (*Glycine max*), anchovy oil (*Engraulis ringers*) and astaxanthin krill oil (*Euphausia superba*). These oil sources were used as they contain a different fatty acid composition allowing feeds to be formulated varying in the amount and profile of these nutrients. One diet acted as a control, with the inclusion of soybean oil only (Control). Other diets contained a combination of soybean oil with fish oil (Fish), or soybean oil with krill oil (Krill). As a result, diets varied in their essential fatty acid (EFA) level and composition, in regard to the dietary content of linoleic acid (LOA, 18:2n-6), linolenic acid (LNA, 18:3n-3), docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). Diets Control, Fish and Krill were evaluated under both ISO (20–26 g/L) and HOS (40–47 g/L) conditions. Two additional diets were designed to contain a combination of soybean oil with an increased (Krill+) and reduced (Krill-) dietary inclusion of krill oil. These diets were evaluated under HOS condition only. The fatty acid composition and sensory characteristics (texture, colour and flavour) of the tail of juvenile *L. vannamei* were evaluated after harvest from HOS culture. A consumer preference assay was carried out with shrimp fed diets Control, Fish, Krill and Krill+.

2.2 | Growth study

Hatchery-reared postlarvae (PL12) were obtained from a commercial hatchery (Sea Life Ltda., Cajueiro da Praia, Brazil) and cultured in an outdoor nursery system for 48 days. Shrimp of 0.65 ± 0.28 g body weight (n = 152) were transferred and stocked in 50 clear-water indoor tanks of 0.5 m^3 at 80 shrimp per tank (140 shrimp/m^2) under a water salinity of 31 ± 1.8 g/L. The tank rearing system adopted is described by Sabry-Neto, Lemos, Raggi, and Nunes (2016).

Seawater at 50 g/L was used to raise water salinity in rearing tanks. Hypersaline water was achieved by first diluting 1 kg of crude sea salt (996.4 g/kg of sodium chloride, Cimsal Indústria Salineira, Mossoró, Brazil) into 100 L of freshwater. This concentrated mix was added to a 20-m^3 header tank with seawater at 31 g/L at quantities needed to achieve the targeted salinity. Water was then allowed to sand filter for 1 week before use. To reduce water salinity, freshwater was added directly into the rearing tanks. The reduction and increase in salinity were achieved gradually at an average of 1.2 ± 0.2 and 1.4 ± 0.2 g L^{-1} day^{-1}, respectively.

Shrimp were first allowed to acclimate feeding on a commercial crumbled shrimp feed (400 g/kg crude protein, Camanutri 40 CR2, InVivo Nutrição e Saúde Animal Ltda., São Lourenço da Mata, Brazil). After 1 week of rearing, water salinity was slowly adjusted to 22.0 ± 0.4 g/L (in 20 tanks) and 41.0 ± 0.4 g/L (in 30 tanks) over a 15-day period. Shrimp were then reared for another 15 days. When animals reached 2.79 ± 0.60 g (n = 1,200), they were captured, individually weighed to 0.01 g (Ohaus Adventurer, Toledo do Brasil, São Paulo, Brazil) and restocked at 40 shrimp per tank (70 shrimp/m^2).

During the study period, shrimp were fed twice a day for 64 days exclusively in feeding trays. Water pH, salinity and temperature were measured daily in each rearing tank. On the 24th and 48th days of rearing, 10 shrimp from each tank were captured, weighed individually to 0.01 g and returned to their respective tank.

Shrimp were individually weighed at stocking and at harvest to determine their initial and final body weight (g), weekly growth rate (g/week), yield (g of biomass gained per m^2) and final survival (%). FCR (food conversion ratio) was calculated based on apparent feed intake (AFI) determined on a dry matter basis according to Nunes, Sá, and Sabry-Neto (2011) and Browdy, Bharadwaj, Venero, and Nunes (2012). AFI was the total amount of feed delivered subtracted from the total amount of feed remains recovered from feeding trays on a dry matter basis. The sum of feed intake per tank was then divided by the number of stocked shrimp in each rearing unit. Water quality management, shrimp feeding and sampling followed a similar protocol as described by Nunes et al. (2011).

2.3 | Feed formulation and preparation

Experimental diets were formulated using a least-cost formulation software (Feedsoft® Professional, Feedsoft Corporation, Richardson, TX, USA). Diets contained a nearly equivalent ingredient and proximate chemical composition (Table 1). Variations in composition were

TABLE 1 Ingredient and proximate composition of experimental diets

	Diets/Composition (g/kg of the diet, as is)				
	Control	Fish	Krill-	Krill	Krill+
Ingredients (g/kg of the diet)					
Soybean meal[a]	350.0	350.0	350.0	350.0	350.0
Wheat flour[b]	300.0	298.7	300.1	299.8	291.5
Poultry by-product meal[c]	105.0	100.0	100.0	100.0	67.5
Anchovy fishmeal[d]	70.6	60.2	75.6	71.6	68.9
Soy protein concentrate[e]	16.6	32.5	16.6	19.1	20.0
Fish oil[f]	–	26.6	–	–	–
Krill oil[g]	–	–	14.5	48.3	55.0
Soybean oil[h]	34.5	10.0	21.2	4.4	3.8
Soybean lecithin[i]	15.0	15.0	15.0	–	–
Cholesterol, 91%[j]	1.3	–	–	–	0.4
Corn gluten meal[k]	–	–	–	–	4.0
DL-methionine[l]	8.0	8.0	8.0	8.0	4.6
Ascorbic acid[m]	2.0	2.0	2.0	1.8	1.3
Others[n]	97.0	97.0	97.0	97.0	97.0
Proximate composition[o] (g/kg of the diet, dry matter basis)					
Moisture	96.7	111.1	98.9	95.6	90.0
Crude protein	354.4	351.8	351.8	353.5	353.1
Ash	89.5	93.7	91.0	91.0	82.3
Crude fibre	9.0	12.0	13.3	13.3	12.0
Crude lipid	94.0	88.8	80.5	80.8	91.3
Nitrogen-free extract	433.1	453.7	463.4	461.4	461.3
Gross energy[p] (kJ/g)	17.9	17.5	17.6	17.6	17.5

[a]Farelo de Soja 46. Bunge Alimentos S.A. (Luis Eduardo Magalhães, Brazil). 447.8 g/kg crude protein (CP), 59.7 g/kg lipid, 64.5 g/kg ash, 54.0 g/kg fibre, 82.2 g/kg moisture.

[b]119.5 g/kg CP, 30.1 g/kg lipid, 5.8 g/kg ash, 0.5 g/kg fibre, 100.0 g/kg moisture.

[c]NORDAL Nordeste Indl. de Derivados Animais Ltda. (Maracanaú, Brazil). 640.4 g/kg CP, 129.2 g/kg lipid, 150.1 g/kg ash, 7.9 g/kg fibre, 96.7 g/kg moisture.

[d]COPEINCA Corporación Pesquera INCA S.A. (Lima, Peru). 641.9 g/kg CP, 62.3 g/kg lipid, 158.3 g/kg ash, 0.9 g/kg fibre, 104.4 g/kg moisture.

[e]Sementes Selecta S.A. (Goiânia, Brazil). 626.4 g/kg CP, 7.7 g/kg lipid, 42.3 g/kg ash, 43.3 g/kg fibre, 82.2 g/kg moisture.

[f]COPEINCA Corporación Pesquera INCA S.A. (Lima, Peru). 980.0 g/kg lipid, 5.18 meq/kg of peroxide, 8.08 mg NaOH per g of acidity.

[g]Astaxanthin oil, Aker Biomarine AS (Oslo, Norway). 795.0 g/kg lipid, 6.0 g/kg cholesterol, <0.001 meq/kg of peroxide, 9.39 mg NaOH per g of acidity.

[h]Bunge Alimentos S.A. (Luis Eduardo Magalhães, Brazil). 996.0 g/kg lipid, 1.80 meq/kg of peroxide, 0.08 mg NaOH per g of acidity.

[i]Courtesy of Cargill Nutrição Animal Ltda. (São Paulo, Brazil). 927.6 g/kg lipid, 61.1 g/kg ash.

[j]Cholesterol SF, Dishman Netherlands B.V. (Veenendaal, Netherlands).

[k]Protenose®, Ingredion Brasil—Ingredientes Industriais Ltda. (Conchal, Brazil). 649.9 g/kg CP, 119.0 g/kg lipid, 15.2 g/kg ash, 13.4 g/kg fibre, 63.3 g/kg moisture.

[l]MetAMINO®, Evonik Degussa Brasil Ltda. (São Paulo, Brazil). DL-Methionine 99%, feed grade. 990 g/kg of methionine, 581 g/kg CP, 5 g/kg ash, 15 g/kg moisture.

[m]Rovimix Stay C® 35%, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). L-ascorbic acid 2-monophosphate, $Na_2Ca_{0.5}C_6H_6O_9P$.

[n]Others included 40.0 g/kg of broken rice[q], 10.0 g/kg of whole squid meal[r], 13.0 g/kg of dicalcium phosphate[s], 10.0 g/kg potassium chloride, 10.0 g/kg common salt, 10.0 g/kg vitamin–mineral premix[t] and 4.0 g/kg of synthetic binder[u].

[o]AOAC (2002) standard methods. Analysed in duplicate.

[p]Parr Bomb calorimeter.

[q]Brasília Alimentos Ltda. (Cruz do Rio Pardo, Brazil). 88.3 g/kg CP, 18.0 g/kg lipid, 13.1 g/kg ash, 2.2 g/kg fibre, 93.3 g/kg moisture.

[r]Hinrichsen Trading S.A. (Santiago, Chile). 688.9 g/kg CP, 53.8 g/kg lipid, 116.4 g/kg ash, 5.1 g/kg fibre, 108.9 g/kg moisture.

[s]Serrana Foscálcio20. Bunge Fertilizantes S/A. (Cubatão, Brazil). 205 g/kg calcium, 202 g/kg total phosphorus, 191 g/kg available phosphorus.

[t]Rovimix Camarao Intensivo. DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). Guarantee levels per kg of product: vitamin A, 1,250,000 IU; vitamin D3, 350,000 IU; vitamin E, 25,000 IU; vitamin K3, 500 mg; vitamin B1, 5,000 mg; vitamin B2, 4,000 mg; vitamin B6; 10 mg; nicotinic acid, 15,000 mg; pantothenic acid, 10,000 mg; biotin, 150 mg; folic acid, 1,250 mg; vitamin 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.

[u]Pegabind™, Bentoli Agrinutrition (TX, USA). Synthetic pellet binder composed of urea formaldehyde.

driven by the different dietary inclusions of the oil sources under evaluation.

Dietary inclusion of soybean meal was fixed at 350 g/kg (as is basis) in all diets. Wheat flour, poultry by-product meal, anchovy fishmeal and soy protein concentrate were used at (mean ± standard deviation) 298 ± 37, 95 ± 15, 69 ± 6 and 21 ± 7 g/kg, respectively. Poultry by-product meal and fishmeal were replaced by soy protein concentrate to allow higher supplementation of oil while maintaining consistent dietary lipid levels. Whole squid meal was fixed at 10 g/kg in all diets to enhance feed attractability. Crude protein content reached a mean of 353 ± 13 g/kg of the diet (dry matter basis).

Diets contained a total crude lipid content of 87 ± 6.2 g/kg. In the Control diet, soybean oil was included at 35 g/kg. In the diet containing 27 g/kg fish oil, inclusion of soybean oil was reduced to 10 g/kg in order to adjust for total dietary lipid content. Similarly, diets Krill-, Krill and Krill+ contained 15, 48 and 55 g/kg of krill oil with soybean oil at 21, 4 and 4 g/kg, respectively. Diets Fish, Krill and Krill+ were designed to contain a greater proportion of DHA and EPA relative to LOA and LNA. Comparatively, diets Control and Krill- showed opposite ratios due to higher dietary inclusion levels of soybean oil.

Dietary levels of cholesterol and phospholipids followed the recommendations made by Gong, Lawrence, Jiang, Castille, and Gatlin (2000). Their levels were adjusted to be nearly similar in each formula. Diets were manufactured with a laboratory extruder following the procedures described by Browdy et al. (2012). During feed preparation, all oils were weighed and included during ingredient mixing, prior to extrusion.

2.4 | Chemical analysis

A total of 200 g of each finished diet was collected for chemical analysis. Shrimp were sampled immediately after harvest; the tail muscle without the carapace was removed, washed and stored under −22°C until analysis. Fatty acid analysis was carried out in finished diets and on the shrimp tail muscle using high-resolution gas chromatography with a flame ionization detection fitted with a capillary GC column (SP™-2560, 100 m × 0.25 mm, Sigma Aldrich Brasil Ltda., São Paulo, Brazil). Lipid extraction was performed following the procedures by Bligh and Dyer (1959). Saponification and methylation of the lipid fraction were carried out according to Hartman and Lago (1973). Total carotenoid was determined through high-performance liquid chromatography (HPLC) according to Passos (2007). Samples of soybean oil, fish oil and krill oil were analysed for peroxide and hydrolytic acidity following AOAC (2002) procedures.

Total fatty acid composition of experimental diets varied according to the type of oil and their dietary inclusion (Table 2). While the levels of polyunsaturated fatty acids (PUFA) were higher in the Control diet, increased levels of highly unsaturated fatty acids (HUFA) were observed in diets containing fish and krill oil, particularly DHA (22:6n-3) and EPA (20:5n-3). The amount of HUFA relative to the total fatty acid content in the Control, Fish, Krill-, Krill and Krill+ diets reached 93.0, 80.1, 29.2, 69.3 and 88.1 g/kg, respectively. Consequently, higher levels of omega-3 fatty acids were observed in diets with higher inclusions of fish and krill oil. Conversely, Control and Krill- diets contained greater amounts of LOA (18:2n-6) compared to other diets due to higher dietary inclusions of soybean oil. Total carotenoid content increased from 13.80 and 14.70 μg/100 μl in Control and Fish diets to 21.40 and 24.70 μg/100 μl in Krill and Krill+ diets, respectively.

TABLE 2 Fatty acid profile (g/kg of total dietary fatty acid) and carotenoid content (μg/100 μl) of experimental diets

Fatty acid (g/kg of total)	Experimental diets				
	Control	Fish	Krill-	Krill	Krill+
12:0	–	–	–	5.9	6.7
14:0	9.8	35.6	54.1	122.9	139.9
16:0	178.4	203.9	208.3	234.9	267.5
18:0	20.7	52.8	47.8	107.3	122.2
16:1n-7	44.5	47.0	38.6	37.6	32.4
18:1n-9	160.3	155.9	152.4	140.7	122.0
18:2n-6	447.1	283.2	336.6	162.1	139.6
18:3n-3	49.1	34.0	39.4	15.2	13.1
20:4n-6	–	3.8	–	–	–
20:5n-3	6.5	50.9	23.3	53.4	68.0
22:6n-3	2.8	25.4	5.9	15.9	18.1
n-3[a]	58.4	110.3	68.6	84.5	99.2
n-6[b]	447.1	287.0	336.6	162.1	139.6
SFA[c]	208.9	292.3	310.2	471.0	536.3
MUFA[d]	204.8	202.9	191.0	178.3	154.4
PUFA[e]	496.2	317.2	376.0	177.3	152.7
HUFA[f]	9.3	80.1	29.2	69.3	86.1
EFA[g]	505.5	393.5	405.2	246.6	238.8
Total carotenoid (μg/100 μl)	13.80	14.70	12.60	21.40	24.70

[a]n-3, 18:3n-3, 20:5n-3, 22:6n-3.
[b]n-6, 18:2n-6, 20:4n-6.
[c]SFA, saturated fatty acids, 12:0, 14:0, 16:0, 18:0.
[d]MUFA, monounsaturated fatty acids, 16:1, 18:1.
[e]PUFA, polyunsaturated fatty acids, 18:2, 18:3.
[f]HUFA, highly unsaturated fatty acids, 20:4, 20:5, 22:6.
[g]EFA, essential fatty acids, 18:2n-6, 18:3n-3, 20:5n-3, 22:6n-3.

2.5 | Sensorial evaluations

A sensorial assessment was made with the tail muscle of shrimp harvested from the HOS tanks in order to evaluate changes in colour, texture and flavour as a result of dietary manipulation. Organoleptic characteristics of shrimp tails were evaluated by the best–worst scaling method (Jaeger, Jørgensen, Aaslyng, & Wender, 2008). Four groups (Control, Fish, Krill and Krill+) of 60 shrimp of 11.4 ± 2.0 g (n = 48) were collected at harvest and stored at −22°C for 1 week. Shell-on, headless shrimp were prepared by first thawing at room temperature. Subsequently, shrimp tails from each dietary treatment were cooked separately for 5 min in a pan containing 1.5 L of freshwater with table salt dissolved at 3.33 g/L. After cooking, water was

drained and each tail group was placed in styrofoam coolers internally coated with aluminium foil.

Twenty non-trained and non-smoking panellists (10 females and 10 males) were recruited. To avoid biased responses on test variables, participants were not informed about dietary treatments. The sensory analysis consisted of four taste sets with three shrimp each, randomized from the different treatments. Each taste set was served at the same time for all panellists. Panellists graded shrimp tail texture, colour and flavour with the following scores: 1, the most preferred sample; -1, the least preferred sample; and 0, when the sample was not chosen. As each sample was assessed three times by each participant among four rounds, sample scores varied between −3 and 3. The sum of all scores from the 20 participants resulted in a final grade for each parameter evaluated. The moulting stage of shrimp used in the sensory evaluation was determined to support possible differences in tail texture. Determination of moult stage was based on the degree of setae development in shrimp uropods (Oliveira-Cesar, Zhao, Malecha, Ako, & Yang, 2006) identified using a 40x magnification microscope.

2.6 | Statistical analysis

Data normality and homogeneity of variance were tested by Kolmogorov–Smirnov and Bartlett's tests prior to statistical analysis. One-way ANOVA followed by two-by-two comparisons with the Tukey's HSD test used to determine the differences between dietary treatments under the same water salinity. Two-way analysis of variance (ANOVA) was used to determine the relationship between dietary treatment and water salinity.

For the sensorial evaluations, descriptive analysis and non-parametric tests were adopted, when data normality was rejected. Consumers' responses for colour, texture and flavour of each sample were subjected in pair to Mann–Whitney *U* tests. Simple linear regressions were used to determine associations between shrimp tail fatty acid composition with flavour preferences. Spearman's correlation was applied to analyse the influence of consumers' responses of each parameter on the response of another. The statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used. The significant level of 5% was set in all statistical analyses.

3 | RESULTS

Over the course of a nine-week feeding period, water pH and temperature did not vary significantly between shrimp raised under ISO (7.27 ± 0.33 pH; 27.3 ± 0.46°C temperature; and 23 ± 1.2 g/L salinity) and HOS (7.37 ± 0.21 pH; 27.5 ± 0.46°C temperature; and 44 ± 2.0 g/L salinity).

Shrimp final survival was in excess of 90% regardless of dietary treatment ($p > .05$, Table 3). Similarly, no significant effect on gained shrimp yield could be attributed to the oil source used or their dietary inclusion level ($p > .05$). However, shrimp achieved a statistically higher survival when fed diets Control, Fish and Krill under HOS salinity compared to ISO. No significant interaction could be established between final shrimp survival and dietary fatty acid composition.

Under ISO, shrimp grew faster, consumed less feed (AFI) and achieved a lower food conversion ratio (FCR) when fed the Krill diet ($p < .05$). These results were also found under HOS, except in regard to weekly shrimp growth ($p > .05$). Under HOS, reducing the dietary inclusion of krill oil (Krill- diet) from 48 to 15 g/kg had no deleterious effect on shrimp performance ($p > .05$), except for final body weight (Figure 1). Conversely, raising the dietary inclusion of krill oil to 55 g/kg significantly deteriorated FCR ($p < .05$). The dietary inclusion of 27 g/kg fish oil (Fish) led to an increase in apparent feed intake (AFI) and FCR under both water salinities when compared to the Control diet ($p > .05$). Two-way ANOVA revealed that dietary treatment (Control, Fish and Krill diets), other than water salinity, had a significant effect on shrimp weekly growth, AFI and FCR. Also, no significant interaction was observed between dietary treatment and water salinity for all parameters evaluated.

Shrimp weighed 2.79 ± 0.60 g at the time of stocking ($p > .05$). After 64 days of rearing, final shrimp body weight under ISO and HOS ranged from 11.12 ± 1.80 (Fish) to 12.03 ± 1.70 g (Krill) and from 10.86 ± 1.86 (Control) to 11.91 ± 2.29 g (Krill), respectively (Figure 1). During the rearing period, shrimp body weight started to differ statistically after 24 days of culture ($p < .05$). A larger body weight was observed for shrimp fed the Krill (6.61 ± 1.01 g) diet compared with those fed the Control (6.06 ± 1.03 g) and the Fish (5.87 ± 0.90 g) diets under ISO condition. At harvest, shrimp fed the diet containing fish oil achieved the lowest body weight ($p < .05$, Figure 1). This was followed by shrimp fed the Control diet.

Comparatively, under HOS condition, significant differences in shrimp body weight were only detected at harvest. Shrimp fed the Control, Fish and Krill- diets showed no differences in final body weight ($p > .05$). However, they weighed significantly less than shrimp fed the Krill and Krill+ diets ($p < .05$, Figure 1). Water salinity had no significant effect on final shrimp body weight when they were fed the diets Fish and Krill ($p > .05$). However, final body weight of shrimp fed the Control diet deteriorated when salinity increased from 23 ± 1.2 to 44 ± 2.0 g/L ($p < .05$).

Chemical analysis revealed that the fatty acid composition of shrimp tails changed in accordance with the dietary oil source fed and their dietary inclusion level (Table 4). Under both water salinity conditions, the dietary inclusion of marine oils (krill and fish oils) increased the amount of highly unsaturated fatty acids (HUFA) in shrimp tails. The levels of HUFA in shrimp tails responded linearly to higher dietary inclusions of krill oil. On the other hand, the dietary inclusion of soybean oil, in the absence of fish and krill oil, increased the content of monounsaturated fatty acids (MUFA) and PUFA of *L. vannamei* tails. While greater amounts of omega-3 (n-3) were observed in shrimp fed diets containing krill and fish oil, n-6 was higher in shrimp fed diets containing soybean oil or a combination of soybean oil and fish oil. The amount of eicosapentaenoic (20:5n3) acid was higher in the tails of shrimp fed diets containing krill oil. However, the content of docosahexaenoic acid (22:6n-3) was higher in diets containing 27 g/kg of fish oil or with higher inclusions of krill oil (55 g/kg).

TABLE 3 Mean final survival (%), final body weight (g), weekly growth (g), gained yield (g/m^2), apparent feed intake (g/shrimp) and food conversion ratio (FCR) of *L. vannamei* after a 64-day rearing period under 23 ± 1.2 g/L (ISO) and 44 ± 2.0 g/L (HOS) water salinity

		Shrimp Final Performance[b]					
Water salinity	Diets[a]	Survival (%)	Final body weight (g)	Growth (g/week)	Yield (g/m^2)	AFI (g/shrimp)	FCR
ISO	Control	92.5 ± 3.5	11.52 ± 1.60 aA	0.96 ± 0.04 a	555 ± 35	18.4 ± 1.0 a	2.32 ± 0.2 a
	Fish	91.8 ± 4.5	11.12 ± 1.84 bA	0.93 ± 0.06 a	533 ± 64	24.3 ± 1.9 b	3.17 ± 0.4 b
	Krill	90.0 ± 7.9	12.03 ± 1.72 cA	1.02 ± 0.05 b	569 ± 73	15.0 ± 0.5 a	1.87 ± 0.2 c
One-way ANOVA[c]		.720	<.0001	.022	.550	<.0001	<.0001
HOS	Control	95.0 ± 2.6	10.86 ± 1.86 aB	0.88 ± 0.09	536 ± 54	15.7 ± 1.7 a	2.05 ± 0.5 ac
	Fish	93.7 ± 4.7	10.96 ± 1.82 aA	0.90 ± 0.08	529 ± 58	22.2 ± 1.3 b	2.98 ± 0.6 b
	Krill-	95.0 ± 4.2	10.88 ± 1.62 a	0.89 ± 0.05	531 ± 28	19.1 ± 1.4 a	2.52 ± 0.3 abc
	Krill	96.2 ± 5.5	11.91 ± 2.29 bA	0.99 ± 0.01	598 ± 123	17.4 ± 3.0 a	2.00 ± 0.5 c
	Krill+	94.1 ± 5.2	11.79 ± 1.92 b	0.98 ± 0.04	555 ± 75	23.2 ± 1.7 b	2.81 ± 0.4 ab
One-way ANOVA		.896	<.0001	.076	.370	.047	.002
Two-way ANOVA[c]		Survival	Body weight	Growth	Yield	AFI	FCR
Diet		.626	<.0001	.012	.203	<.0001	<.0001
Salinity		.041	.002	.081	.937	.564	.409
Diet × salinity		.756	.050	.645	.709	.278	.444

[a]Control (9.3 g/kg HUFA relative to the total dietary fatty acid composition), diet containing 35 g/kg soybean oil; Fish (80.1 g/kg HUFA), 27 g/kg fish oil with 10 g/kg soybean oil; Krill- (29.2 g/kg HUFA), 15 g/kg krill oil with 21 g/kg soybean oil; Krill (69.3 g/kg HUFA), 48 g/kg krill oil with 4 g/kg soybean oil; Krill+ (88.1 g/kg HUFA), 55 g/kg krill oil with 4 g/kg soybean oil.

[b]Yield, gained shrimp yield (g of biomass per m^2); AFI, apparent feed intake (g of feed consumed per stocked shrimp); FCR, food conversion ratio.

[c]Comparisons between diets Control, Fish and Krill.

Columns with similar letters indicate non-statistically significant differences within each salinity range at α = .05 according to Tukey's HSD. Each value refers to a mean of six to seven rearing tanks.

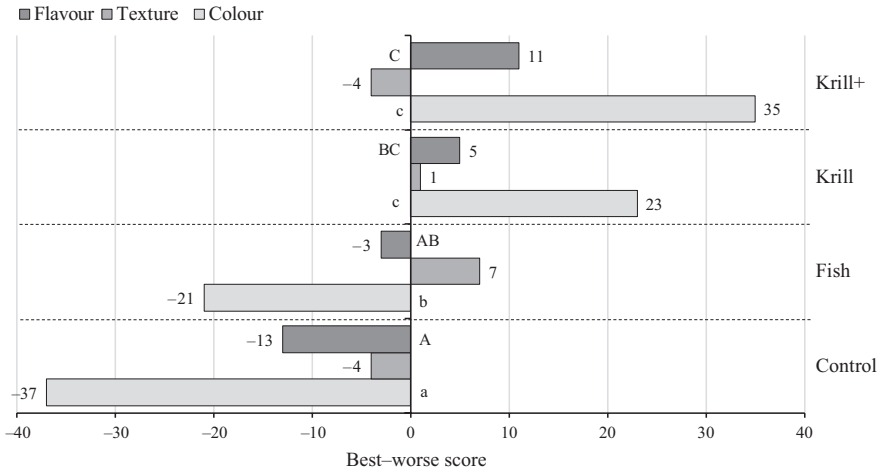

FIGURE 1 Best–worse (B-W) scores for consumer preference on shrimp colour, texture and flavour. Values represent the sum of B-W evaluations of 20 panellists (n = 60) for each sample. Different lowercase and uppercase letters indicate statistically significant differences at the α = .05 level by Mann–Whitney U test for shrimp colour and flavour, respectively. Shrimp were raised under 44 ± 2.0 g/L water salinity for 64 days and fed diets containing different oil sources

Tails of shrimp fed diets containing either 48 or 55 g/kg of krill oil were the most preferred (65 and 50%) by consumers in regard to colour, with the highest best–worse (B-W) scores (p < .05, Figure 1). Comparatively, tails of shrimp that had been fed the Control diet scored the lowest preference (7%) in terms of colour, followed by those fed diets with fish oil (12%, p < .05). Consumers' preference for shrimp tail texture was equally distributed among dietary treatments. Thus, B-W scores for this parameter were not statistically different (p > .05). Shrimp moulting stages were not homogenous among dietary treatments (Table 5). This may have affected consumers' preference for this attribute.

In terms of tail flavour, the greatest consumers' preference (45%) was observed for those shrimp that had been fed diets containing higher levels of krill oil (Krill+). However, preference (30%) and B-W scores were not different from those that had been fed Krill (p > .05). The least preferred tails, according to consumers, were those that had been fed diets containing soybean oil (25%), followed by the ones fed

TABLE 4 Fatty acid composition (g/kg of the total fatty acid content) of the tail muscle of juvenile *L. vannamei* raised for 64 days under two water salinities fed diets containing different oil sources. ISO, 23 ± 1.2 g/L; and HOS, 44 ± 2.0 g/L salinity

	Diets[a]/ISO			Diets[a]/HOS				
Fatty acid (g/kg of total)	Control	Fish	Krill	Control	Fish	Krill-	Krill	Krill+
12:0	–	3.9	7.6	–	–	–	–	8.8
14:0	262.8	251.8	238.8	246.3	290.3	257.1	271.6	271.8
16:0	8.5	8.6	29.1	4.8	14.6	8.1	30.2	29.0
17:0	103.9	112.1	102.9	115.9	120.4	121.2	109.8	102.5
18:0	113.0	112.6	114.2	110.5	116.2	106.5	129.4	117.9
16:1n-7	6.7	8.0	10.2	6.0	9.9	6.4	12.1	10.4
18:1n-9	273.1	146.4	116.3	269.4	149.3	205.6	111.0	115.3
20:1n-11	20.3	15.5	9.2	18.0	–	16.6	7.6	7.4
18:2n-6	12.1	6.9	6.7	13.0	6.3	8.5	5.4	–
18:3n-3	–	4.1	–	–	–	–	–	–
20:4n-6	16.6	17.9	11.6	13.3	17.4	11.1	9.4	12.0
20:5n-3	60.6	124.2	149.7	66.0	101.0	104.0	115.0	139.2
22:6n-3	36.9	71.3	65.5	35.3	53.4	43.7	42.2	56.6
n-3[b]	97.5	199.6	215.2	101.3	154.4	147.7	157.2	195.8
n-6[c]	28.7	24.8	18.3	26.3	23.7	19.6	14.8	12.0
SFA[d]	488.2	489.0	492.6	477.5	541.5	492.9	541.0	530.0
MUFA[e]	300.1	169.9	135.7	293.4	159.2	228.6	130.7	133.1
PUFA[f]	12.1	11.0	6.7	13.0	6.3	8.5	5.4	–
HUFA[g]	114.1	213.4	226.8	114.6	171.8	158.8	166.6	207.8
EFA[h]	109.6	206.5	221.9	114.3	160.7	156.2	162.6	195.8

[a]Control (9.3 g/kg HUFA relative to the total dietary fatty acid composition), diet containing 35 g/kg soybean oil; Fish (80.1 g/kg HUFA), 27 g/kg fish oil with 10 g/kg soybean oil; Krill- (29.2 g/kg HUFA), 15 g/kg krill oil with 21 g/kg soybean oil; Krill (69.3 g/kg HUFA), 48 g/kg krill oil with 4 g/kg soybean oil; Krill+ (88.1 g/kg HUFA), 55 g/kg krill oil with 4 g/kg soybean oil.
[b]n-3, 18:3n-3, 20:5n-3, 22:6n-3.
[c]n-6, 18:2n-6, 20:4n-6.
[d]SFA, saturated fatty acids, 12:0, 14:0, 16:0, 18:0.
[e]MUFA, monounsaturated fatty acids, 16:1, 18:1, 20:1.
[f]PUFA, polyunsaturated fatty acids, 18:2, 18:3.
[g]HUFA, highly unsaturated fatty acids, 20:4, 20:5, 22:6.
[h]EFA, essential fatty acids, 18:2n-6, 18:3n-3, 20:5n-3, 22:6n-3.

fish oil (30%). The latter was not statistically different in terms of flavour from those that had been fed Krill ($p > .05$).

Consumers' scores for shrimp tail flavour were positively correlated with the levels of palmitoleic (16:1n-7, $r^2 = .91$, $p < .05$) and eicosapentaenoic acids (20:5n-3, $r^2 = .91$, $p < .05$). There was a significant, but negative correlation between B-W scores for shrimp tail flavour and the levels of linolenic (18:3n3, $r^2 = -.94$, $p < .05$), linoleic (18:2n6, $r^2 = -.85$, $p < .05$) and stearic ($R^2 = -.63$, $p < .05$) acids observed in the tails.

4 | DISCUSSION

In the present study, the dietary fatty acid composition and source of oil had no effect over shrimp final survival. However, we have found that a lower water salinity (ISO, 23 ± 1.2 g/L) was more negative to shrimp survival than a higher one (HOS, 44 ± 2.0 g/L). These findings are in accordance with other investigations. González-Félix, Gatlin, Lawrence, and Pérez-Velázquez (2002a) raised *L. vannamei* of 3.57 ± 0.23 g for 6 weeks under 24.8 g/L salinity. Shrimp were fed diets containing different oil sources (coconut, soybean, linseed, peanut and menhaden oils) at 50 g/kg dietary inclusion with and without 31 g/kg lecithin supplementation. At harvest, the authors reported no significant differences in final shrimp survival ($p > .05$). In another work, González-Félix, Gatlin, Lawrence, and Pérez-Velázquez (2002b) reported a detriment in final shrimp survival when animals were fed diets with excessive levels of DHA (81.2 and 98.8 g/kg of 22:6n-3) under 25.0 ± 0.7 g/L for 6 weeks. Compared to González-Félix et al. (2002b), our diets contained DHA levels below 25.4 g/kg (on a total dietary fatty acid basis) with no interaction with final shrimp survival. Therefore, in our work, it is unlikely that any effect on final survival was related to the dietary fatty acid composition other than

TABLE 5 Relative distribution (%) of moulting stages for juvenile *L. vannamei* used in the consumer preference assay

	Diets[b]/Distribution (%)			
Moulting stage[a]	Control	Fish	Krill	Krill+
A (early postmoult)	8.3 (1)	–	8.3 (1)	16.7 (2)
B (late postmoult)	16.7 (2)	16.7 (2)	8.3 (1)	25.0 (3)
C (intermoult)	25.0 (3)	8.3 (1)	8.3 (1)	–
D_0 (onset of premoult)	–	16.7 (2)	33.3 (4)	16.7 (2)
D_1 (early premoult)	33.3 (4)	16.7 (2)	41.7 (5)	8.3 (1)
D_2 (intermediate premoult)	8.3 (1)	8.3 (1)	–	16.7 (2)
D_3 (late premoult)	8.3 (1)	33.3 (4)	–	16.7 (2)
E (moult)	–	–	–	–

[a]According to Oliveira-Cesar et al. (2006).

[b]Control (9.3 g/kg HUFA relative to the total dietary fatty acid composition), diet containing 35 g/kg soybean oil; Fish (80.1 g/kg HUFA), 27 g/kg fish oil with 10 g/kg soybean oil; Krill- (29.2 g/kg HUFA), 15 g/kg krill oil with 21 g/kg soybean oil; Krill (69.3 g/kg HUFA), 48 g/kg krill oil with 4 g/kg soybean oil; Krill+ (88.1 g/kg HUFA), 55 g/kg krill oil with 4 g/kg soybean oil.

Values in parentheses indicate the number of shrimp in the respective moulting stage.

the salinity itself. Results of Hurtado et al. (2006) also support our findings that in low, rather than in high salinity, shrimp survival was found to be decreased. The authors raised *L. vannamei* of 3.5 ± 0.5 g with diets containing 15 ± 0.1 and 150 ± 0.6 g/kg DHA. Shrimp were fed for 21 days under low (5 g/L), medium (30 g/L) and high (50 g/L) salinities. At harvest, there was no significant effect on final shrimp survival associated with dietary DHA ($p > .05$). However, survival of shrimp raised under 5 g/L salinity was significantly lower than those under higher salinity levels (30 and 50 g/L). Maicá, Borba, Martins, and Wasielesky-Jr (2014) evaluated the effect of three water salinities (4, 16 and 32 g/L) over the growth performance and survival of *L. vannamei* raised for 36 days under 140 animals per m^2. The authors found a significant improvement in final shrimp survival, from 74.8 ± 4.93 and 72.8 ± 4.65 to 88.3 ± 0.58%, respectively, with a higher water salinity.

In the present work, the dietary oil source and their inclusion level significantly affected shrimp weekly growth and final body weight. The dietary inclusions of 48 and 55 g/kg of krill oil enhanced shrimp weekly growth under ISO (23 ± 1.2 g/L salinity), while final body weight was increased under both salinities evaluated. Under HOS, final shrimp body weight was significantly enhanced, when shrimp were fed diets containing increased levels of krill oil (Krill and Krill+). This appears to be related to both the dietary oil source and the amount of HUFA in the diets. While these latter diets contained a higher HUFA content (69.3 and 86.1 g/kg, respectively) compared to Control and Krill- diets (9.3 and 29.2 g/kg, respectively), levels were not different from the diet containing fish oil (80.1 g/kg HUFA). Under 50 g/L salinity, Hurtado et al. (2006) did not observe any differences in shrimp growth when fed HUFA-enriched diets (29 versus 340 g/kg HUFA). However, the dietary levels of HUFA in Hurtado et al.'s (2006) work are much higher than the one adopted in the present study. Toledo, Silva, Vieira, Mouriño, and Seiffert (2016) investigated the effect of increasing the dietary lipid level on the growth and survival of juvenile *L. vannamei* raised under a biofloc condition with a constant water salinity of 33 g/L. The authors achieved dietary lipid levels of 87.8, 96.5 and 104.8 g/kg through a higher inclusion of soybean, poultry and corn oils. While shrimp growth was unaffected by higher lipid levels, final survival dropped significantly from 92.5 ± 3.5 and 91.0 ± 2.5% to 78.8 ± 5.5%, respectively.

Arachidonic (20:4n-6), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids have a greater nutritional value to *L. vannamei* than linoleic (18:2n-6) and linolenic (18:3n-3) acids (González-Félix, Gatlin, Lawrence, & Perez-Velazquez, 2003). However, in our work, we could not demonstrate the benefit of fish oil over soybean oil, regardless of water salinity. This contrasts with other work that concluded that fish oil is a better oil source to *L. vannamei* than soybean oil. González-Félix, Lawrence, Gatlin, and Pérez-Velázquez (2002) have found a higher instantaneous growth rate (IGR) for *L. vannamei* fed diets containing menhaden oil (*Brevoortia tyrannus*) compared to shrimp fed soybean oil. Lim, Ako, Brown, and Hahn (1997) reared juvenile *L. vannamei* under 35 g/L salinity for 70 days. The authors reported a higher final survival (81.7 versus 60.0%, respectively) and body weight (4.0 versus 2.6 g, respectively) in shrimp fed menhaden oil compared to soybean oil. However, our diet contained only 27 g/kg of fish oil compared to 50 and 65 g/kg in diets formulated by González-Félix, Lawrence et al. (2002) and Lim et al. (1997), respectively. These higher dietary inclusions enhanced the total HUFA content, most likely improving shrimp performance. In our study, the source of oil and their dietary inclusion level did not significantly improve shrimp final yield under both water salinities. In contrast, Hurtado et al. (2006) observed a significant influence of water salinity on the final shrimp biomass after a 21-day culture of *L. vannamei*. The authors reported that shrimp reared under 30 g/L salinity achieved a higher biomass compared to those reared under 5 and 50 g/L, regardless of the dietary level of HUFA.

The increase in water salinity in shrimp culture generally increases food conversion ratio (FCR) and feed intake (AFI; Hurtado et al., 2006). In our study, although feed intake was measured indirectly (based on feed delivered minus feed recovered from feeding trays), no effect of water salinity could be associated with these parameters. Nevertheless, the dietary inclusion of 48 g/kg krill oil significantly improved FCR and AFI compared to a diet with 27 g/kg fish oil, under both ISO and HOS. No differences in FCR and AFI could be observed between shrimp fed the diet containing 48 g/kg krill and the diet with 35 g/kg soybean oil. Similarly, González-Félix, Lawrence et al. (2002) did not detect differences in FCR of *L. vannamei* fed diets containing coconut, soybean, linseed, peanut or menhaden oil under 24.8 g/L salinity after 56 days of rearing.

González-Félix et al. (2002b) observed an enhancement in IGR when shrimp were fed DHA- or n-3 HUFA-enriched diets. Their diets contained a total n-3 content ranging from 38.8 to 99.6 g/kg. The authors suggested that no further levels of n-3 are required by

L. vannamei. In our study, an enhancement of shrimp growth and final body weight was only achieved when total dietary n-3 levels reached 84.3 g/kg (Krill diet). When results of the present work are compared to those conducted with *P. monodon* (Glencross & Smith, 1997, 1999, 2001a, 2001b; Glencross, Smith, Thomas, & Williams, 2002a, 2002b), it is possible that n-3 requirements are lower in *L. vannamei*. Glencross et al. (2002a) achieved a maximum growth in *P. monodon* fed a total dietary lipid content of 75.0 g/kg, of which 30.0–43.0 g/kg was essential fatty acids (EFA, sum of 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3) respectively. In our work, an enhanced performance was achieved when *L. vannamei* was fed a dietary lipid content ranging from 80.8 to 91.3 g/kg, of which 19.9–20.3 g/kg was EFA (Krill and Krill+ diets, respectively). However, the dietary EPA (20:5n-3) and DHA (22:6n-3) ratios in our diets (Krill, 3.35:1; and Krill+, 3.75:1) were different from the 1:1 ratio recommended for the tiger shrimp (Glencross & Smith, 1999, 2001a). Similarly, while LOA (18:2n-6) and LNA (18:3n-3) ratios suggested for *P. monodon* are in the range of 0.66:1 (Glencross & Smith, 1999, 2001a), our best-performing diets reached 10.66:1 (diets Krill and Krill+). In our study, regardless of the EPA/DHA ratio, a minimum dietary EPA content of 53 g/kg was sufficient to avoid loss in shrimp performance in salinities above 40 g/L.

Overall, shrimp fed diets supplemented with astaxanthin krill oil achieved an enhanced performance in terms of growth and final body weight under both water salinities (23 ± 1.2 and 44 ± 2.0 g/L). This can also be associated with antioxidants present in the oil, such as astaxanthin. Diets with the highest inclusion of krill oil (Krill and Krill+) showed the highest total carotenoid concentrations (21.40 and 24.70 μg/100 μl, respectively) compared to other diets (13.80, 14.70 and 12.60 μg/100 μl in Control, Fish and Krill-, respectively). These compounds confer higher stability to the feed lipids and add beneficial effects for shrimp as a natural antioxidant (Fricke, Gercken, Schreiber, & Oehlenschläger, 1984; Grynbaum et al., 2005; Niki, 1987).

In this work, higher concentrations of EPA and DHA in shrimp tails were found in animals fed the HUFA-rich diets (Fish, Krill and Krill+ diets) compared to Control and Krill- diets. This result corroborates with observations made for *M. japonicus* (Guary, Kayama, Murakami, & Ceccaldi, 1976), *P. monodon* (Catacutan, 1991; Kumaraguru vasagam, Ramesh, & Balasubramanian, 2005) and *L. vannamei* (González-Félix, Lawrence, Gatlin, & Pérez-Velázquez, 2003; González-Félix, Lawrence et al., 2002; González-Félix, Gatlin et al., 2003; Hurtado et al., 2006). Modification of the fatty acid profile of aquaculture products by handling dietary lipid sources has been extensively demonstrated in several previous studies (see reviews by Turchini, Torstensen, & Ng, 2009 and Glencross, 2009).

A particular observation from the present study was the influence of water salinity on the fatty acid profile of *L. vannamei* tails. When comparing the composition of the animals fed the same diet at ISO (23 ± 1.2 g/L) and HOS (44 ± 2.0 g/L), a marked reduction in the levels of EPA and EFA could be observed in the Fish and Krill groups reared under HOS when compared to ISO. EFA levels were found to be reduced by over 20% in tails from shrimp fed the Fish and Krill treatments. An opposite trend was observed in animals fed the Control diet, which increased tail EFA levels by 4.3% at HOS. In the present study, the higher performance of Krill and Krill+ fed shrimp may have led to the reduced concentration of EFA found in muscles of shrimp reared in hypersaline conditions (HOS). This may indicate an increased metabolic utilization of these fatty acids in these groups, both for increased energy utilization and for maintenance of osmoregulation and homeostatic processes.

Consumer acceptance by colour and flavour was markedly superior for tails from shrimp fed diets containing astaxanthin krill oil. Diets Krill and Krill+ contained the highest concentrations of astaxanthin pigment in comparison with Control and Fish oil diets reflecting their lipid source. In a review conducted by Diler and Dilek (2002), the authors suggested for pigmentation enhancement a dietary astaxanthin supplementation between 75 and 100 mg/kg for 3 months prior to harvesting or 40 to 50 mg/kg for a six-month period. The present results allow for a reflection on this recommendation, considering that consumers were able to distinguish and express significant preferences for tails from shrimp fed diets containing only slight differences in total carotenoid concentrations.

In the present study, differences in shrimp moulting phases could have been the main determinant in the texture preference responses, making the evaluation more subjective considering the use of untrained tasters and shell-on shrimp. Thus, to establish more reliable data for consumer preference of shrimp tail texture, use of shrimp at the same moulting stage or peeled shrimp should be considered in future studies. Further research establishing consumer responses to the different moult stages and correlating them with high-precision instrumental analyses will assist towards a better understanding of the influence of this variable on shrimp acceptance.

The different oil sources utilized generated distinct shrimp tail fatty acid compositions, which were highly correlated with the preference responses from the panellists. Shrimp tail acceptability for shrimp fed diets with astaxanthin krill oil was positively correlated (R^2 = .82), whereas responses for the Control diet (soybean oil) showed a high negative correlation (R^2 = −.84). These results are in agreement with Waagbø, Sandnes, Torrissen, Sandvin, and Lie (1993) who found a positive association of the organoleptic characteristics in salmon fillets with increased n-3 fatty acid levels.

5 | CONCLUSIONS

Based on the data obtained in the present study, the importance of the dietary supplementation of EFA was clear, especially when *L. vannamei* is farmed in salinities near or above 40 g/L. A minimum total dietary n-3 content of 84.3 g/kg is required to avoid suppressed growth under these conditions. This can be applied in commercial feed formulations for *L. vannamei* and guide future studies on essential fatty acid ratios. However, the exact mechanism behind the positive effects of the dietary inclusion of astaxanthin krill oil needs to be addressed in future studies. The results further show that the source of oil used in the diets, as well as their respective concentrations of EFA and total carotenoid content, can significantly affect the sensory characteristics

of shrimp, directly impacting on consumer acceptance. The methodology used in this study has high discriminative power, but it was not able to measure in precise terms, the intensity of the variation between the experimental groups. Further studies are needed for the precise and economic establishment of dose–response relationships on shrimp response and consumer perception.

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