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Effect of dietary graded levels of astaxanthin krill oil and high protein krill meal on the growth performance and stress resistance of postlarval *Litopenaeus vannamei* under hyper-intensive nursery culture

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Abstract

This study evaluated the effect of graded dietary levels of astaxanthin krill oil (AKO) and high protein krill meal (HPK) on the growth performance and stress resistance of postlarval (PL) *Litopenaeus vannamei*. Shrimp of 3.6–2.5 mg body weight (BW) were stocked in outdoor and indoor tanks at 2,371–2,504 PLs m^{-3}, respectively. Diets contained 10.0, 30.0 and 50.0 g/kg AKO (outdoor) and 30.0, 50.0 and 70.0 g/kg AKO with 80.0 g/kg HPK (indoor). After 52 and 41 days of nursery in outdoor and indoor tanks, no statistical effect from the inclusion of AKO and AKO with HPK was observed on final mean survival (84.0%–91.8%), gained yield (1,568–1,611 g/m^3), daily growth (14.2–14.2 mg/day) and feed conversion ratio (1.70–0.89), respectively. However, final shrimp BW was significantly improved with 50.0 g/kg AKO (775 mg) and 30.0 g/kg AKO with 80.0 g/kg HPK (629 mg), respectively, compared with control groups (711 and 567 mg). Resistance to osmotic and thermal stress was significantly increased with 10.0 g/kg AKO. Enhancement in growth performance and resistance may be related to a higher supply of dietary EPA, DHA and astaxanthin resulting from the inclusion of AKO and HPK.

KEYWORDS

astaxanthin, high-protein krill meal, krill oil, *Litopenaeus vannamei*, nursery, postlarval, shrimp

1 | INTRODUCTION

Shrimp grow-out has traditionally been carried out in a single phase by direct stocking of hatchery-reared postlarvae (PL) in ponds. However, in recent years, several farms have engaged in a two- or three-stage culture combining hyper-intensive nursery systems with semi-intensive or intensive pond rearing (Browdy et al., 2016; Nunes, 2019). This culture strategy has been shown to provide significant advantages in production, including full compensatory growth after pond stocking (Wasielesky et al., 2013), improved shrimp survival, and greater size uniformity at harvest (Yta et al., 2004).

Nursery represents an intermediate culture phase between the hatchery and grow-out which lasts from 10 to 60 days. While pond-based nurseries of 0.5–1.5 ha in area are still used, hyper-intensive nursery is mostly carried out in above-ground round tanks of 50–200 m^3 and (or) in plastic-lined rectangular tanks or raceways of 300–7,500 m^3, enclosed or not in plastic greenhouses (Browdy et al., 2016; Nunes, 2019; Samocha et al., 2004). The nursery stage usually starts with 9–12-day old PL stocked under 500–5,000 to PLs m^{-3} and ends with juveniles between 300 mg and 3 g of body weight (BW) with a final yield of 1–3 kg/m^3 (Arias-Moscoso et al., 2018; Correia et al., 2014; Samocha et al., 2004; Tierney et al., 2020).

Water quality is controlled through carbon supplementation to promote total ammonia nitrogen (TAN) assimilation by bacteria (Ferreira et al., 2020; Khanjani et al., 2017; Samocha et al., 2007); use of settling chambers (Ray et al., 2010) to keep total suspended solids below 600 mg/L (Schveitzer et al., 2013); with strong aeration and water mixing to reach near saturation in dissolved oxygen (DO) with limited water discharge (Arias-Moscoso et al., 2018; Mishra et al., 2008; Panigrahi et al., 2020; Samocha et al., 2007).

Aggressive feeding programs are deployed during nursery that combine a high feeding frequency with nutrient-dense PL diets (Samocha et al., 2004, 2017; Schveitzer et al., 2017). To address the higher stress to animals because of increased stocking density, organic water loading and handling, as well as to optimize growth, various dietary supplements can be used, such as algae paste, Artemia (Zelaya et al., 2007), probiotics (Arias-Moscoso et al., 2018) and astaxanthin (Liu et al., 2018).

In previous studies, the dietary supplementation of astaxanthin krill oil (AKO), a source of omega-3 polyunsaturated fatty acids (n-3 PUFAs) and the antioxidant astaxanthin, has been shown to improve the growth performance of juvenile whiteleg shrimp, *Litopenaeus vannamei*, farmed under high salinity (Castro et al., 2018; Rufino et al., 2020). In this work, we investigated the effects of dietary graded levels of AKO and/or a high-protein krill meal (HPK) on the survival, growth performance and osmotic and thermal stress resistance of PL *L. vannamei* reared under hyper-intensive nursery culture conditions.

2 | MATERIAL AND METHODS

2.1 | Rearing systems, water preparation and management

Two consecutive nurseries were carried out in this study adopting different rearing systems. The 1st shrimp nursery was conducted in 40 circular outdoor tanks of 1.5 m^3 (1.70 m^2 bottom area × 0.88 m height). Tanks were independent and enclosed under a greenhouse using a 70% dark shading net, but were kept exposed to sunlight and rainfall. The system operated under minimum-water exchange. Water was added only to compensate losses due to evaporation. In the 2nd nursery, shrimp were reared under 50 indoor tanks of 0.5 m^3 (0.57 m^2 bottom area × 0.56 m height) completely housed under a roof with a 12-hr artificial light cycle starting at 05:30 a.m. The indoor rearing system also operated with independent tanks, but under increased water transparency, and limited algal growth. These conditions provided a greater control over environmental variables compared with the outdoor system. Water was exchanged on a weekly basis at 10% of total water volume.

There was no water interexchange between tanks in both systems. Sugar-cane molasses were applied once a week to control total ammonia nitrogen (TAN) and nitrite concentrations (Samocha et al., 2007). Tanks were equipped with air-diffused aeration provided by blowers connected to PVC lines. Air was delivered to the bottom of each tank by a 0.5-m aeration tubing (Aero-Tube™, Tekni-Plex Aeration, Austin, Texas, USA). Aeration was supplied to nearly saturate water with DO during the complete rearing cycle.

Water preparation consisted of filling 90% of total tank volume with sand-filtered seawater and the remaining with matured water from a shrimp nursery tank. Autotrophic nitrifying bacteria development was initially promoted through supplementation of 31 g m^{-3} day^{-1} (as-is basis) of ground shrimp feed (minimum of 350 g/kg CP) with 23 g m^{-3} day^{-1} of liquid sugar-cane molasses over 4 days. An additional 3 days were allowed for water mixing with strong aeration before shrimp stocking.

Water quality parameters (i.e. pH, temperature and salinity) were measured once daily starting at 09:00 a.m. in all rearing tanks. TAN, nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were measured in outdoor tanks three times during the culture period with a visible spectrophotometer (DR 2800 Spectrophotometer, Hach Company, Loveland, USA). Water samples were tested individually for nitrogenous compounds from four outdoor tanks selected randomly from each treatment for a total of 20 readings per sampling day (culture days 14, 28 and 48). Water was always sampled from the water column. No monitoring of N compounds was carried out in the indoor tanks since they were kept within acceptable levels through water exchange. Water alkalinity was kept above 120 mg/L of $CaCO_3$ through applications of sodium bicarbonate.

2.2 | Astaxanthin krill oil and diets

AKO (Qrill™ AstaOmega Oil, Aker BioMarine Antarctic AS, Lysaker, Norway) used in this study was obtained during processing of Antarctic krill, *Euphausia superba*. The analysed fatty acid composition (Table 1) was as follows: 420 g/kg saturated fatty acids (SFA), 377 g/kg monosaturated fatty acids (MUFA) and 35 g/kg PUFA. Arachidonic (20:4n-6, ARA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids reached < 1, 54, and 12 g/kg, respectively. The AKO contained 930 mg/kg of astaxanthin esters.

A total of nine nursery diets were designed for this study (Table 2), split into two sets. The 1st set of four diets were used in the outdoor tank system. First, a control diet (AKO0) was designed without any inclusion of AKO. Other diets contained 10.0 (AKO1), 30.0 (AKO3) and 50.0 g/kg (AKO5) AKO included at the cost of soybean oil and soy lecithin oil to keep them isocaloric and balanced for total phospholipid content. Salmon meal was fixed at 180.0 g/kg in all formulas, along with 44.5 g/kg of poultry viscera and bone meal. Other sources of protein included 203.9 g/kg of soybean meal (SBM), 100.0 g/kg of wheat gluten meal and 80.0 g/kg of soy protein concentrate (SPC).

The 2nd set of five diets was used in the indoor tank system to evaluate the combined effect of AKO and HPK (Qrill™ High Protein meal, Aker BioMarine Antarctic AS, Lysaker, Norway). HPK partially replaced salmon meal, while AKO substituted salmon oil (Table 2) and soy lecithin oil. Initially, a negative control diet (AKO0HPK0) was formulated with 298.0 g/kg of salmon meal, 189.8 g/kg of SBM, 70.0 g/

TABLE 1 Fatty acid profile (g/kg of total dietary fatty acids, dry matter basis) of the AKO and salmon oil used in this study

	Composition (g/kg)	
Fatty acid	AKO	Salmon oil
12:0	–	1
14:0	190	24
15:0	–	2
16:0	215	120
17:0	–	3
18:0	15	37
20:0	<1	3
21:0	–	6
22:0	<1	8
24:0	–	1
17:1	–	2
16:1n-7	119	32
18:1(n-9) + (n-7) + (n-5)	237	397
20:1(n-9) + (n-7)	17	21
22:1(n11) + (n-9) + (n-7)	4	3
24:1n-9	<1	3
16:2n-4	12	–
16:3n-4	3	–
18:2n-6	12	188
18:3n-6	2	2
20:2n-6	<1	10
20:3n-6	1	1
20:4n-6	<1	3
22:2n-6	–	1
22:4n-6	<1	–
18:3n-3	4	55
18:4n-3	19	–
20:3n-3	1	8
20:4n-3	2	–
20:5n-3	54	–
21:5n-3	2	–
22:5n-3	1	33
22:6n-3	12	41
n-3[1]	95	137
n-6[2]	15	201
SFA[3]	420	203
MUFA[4]	377	460
PUFA[5]	35	186
HUFA[6]	70	76
Peroxide value (meq peroxide kg[6] oil)	<2.0	na[7]
Astaxanthin esters (mg/kg)	930	na

[1]n-3, 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3.

[2]n-6, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6.

[3]SFA, saturated fatty acids, 14:0, 16:0, 18:0, 20:0, 22:0.

[4]MUFA, monounsaturated fatty acids, 16:1, 20:1, 22:1, 24:1.

[5]PUFA, polyunsaturated fatty acids, 18:2n-6, 18:3n-6.

[6]HUFA, highly unsaturated fatty acids, 20:4n-6, 20:5n-3, 22:6n-3.

[7]na, not available.

kg of SPC and 50.0 g/kg of wheat gluten meal. From the AKO0HPK0 diet, four other diets were prepared by partially replacing salmon meal for 80.0 g/kg of HPK, including a positive control (KO0HPK8) without AKO. The three additional diets were supplemented with graded levels of AKO at 30.0 (AKO3HPK8), 50.0 (AKO5HPK8) and 70.0 g/kg (AKO7HPK8) at the cost of salmon oil and soy lecithin oil. Soybean oil was included to balance for total dietary lipid content. A total of 10 tanks were assigned for each dietary treatment in the outdoor and indoor tanks.

Diets were laboratory-manufactured according to the methods described in Nunes et al. (2011). Pellets of 2.0 mm in diameter by 5 mm in length were ground to obtain crumbled particles. Crumbles were separated into different particle sizes using a mechanical shaker equipped with sieves (MA750, Marconi Equipamentos para Laboratórios Ltda., Piracicaba, São Paulo Brazil) in sizes ranging from less than 250 μm to more than 1 mm.

Finished diets were chemically analysed (AOAC, 2005). 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 analyse crude protein (CP, AOAC 968.06), while ether extract 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 fibre by enzymatic-gravimetric determination (AOAC 992.16). Amino acid (AA) and fatty acid (FA) compositions were determined using high-performance liquid chromatography (Hagen et al., 1993; White et al., 1986) and high-resolution gas chromatography (GC) with a flame ionization detection fitted with a capillary GC column, respectively.

The 1st and 2nd set of diets contained 407.8 ± 1.9 (mean ± standard deviation, *SD*) and 412.9 ± 3.2 g/kg of CP (as-is basis) and 88.5 ± 12.5 and 84.6 ± 8.7 g/kg of total lipids, respectively (Table 2). Mean dietary lysine (Lys), methionine (Met) and M + C ranged from 20.7 ± 0.2 to 22.1 ± 0.5 g/kg Lys, 8.1 ± 0.1 to 8.7 ± 0.2 g/kg Met and 14.4 ± 0.1 to 14.2 ± 0.5 g/kg M + C, respectively (Table 3). While there was a drop in the total amount of PUFA with increasing levels of AKO, the levels of HUFA, particularly EPA and DHA improved, from 1.2 and 1.9 g/kg in the AKO0 diet to 4.9 and 3.1 g/kg in the AKO5 diet, respectively (Table 4). HUFA content increased in the 2nd set of diets compared to the 1st set due to a higher inclusion of salmon meal, HPK and AKO. EPA levels progressively increased from 2.5 g/kg in diet AKO0HPK0 to 7.9 g/kg in diet AKO7HPK8. On the other hand, replacement of salmon meal for HPK without the inclusion of AKO dropped the levels of DHA from 3.6 g/kg in the AKO0HPK0 to 2.9 g/kg in diet AKOHPK8. DHA levels were progressively recovered by graded dietary inclusions of AKO, reaching the highest level of 3.8 g/kg when AKO reached 70.0 g/kg (AKO7HPK8).

2.3 | Shrimp stocking

The shrimp species used in this study was the Pacific whiteleg shrimp, *L. vannamei*, purchased as PL from two commercial

TABLE 2 Ingredient and proximate composition (g/kg, as-is basis) of nursery diets

	Outdoor tanks				Indoor tanks				
	Astaxanthin krill oil (AKO)				Astaxanthin krill oil (AKO) & high-protein krill meal (HPK)				
Ingredients	AKO0	AKO1	AKO3	AKO5	AKO0HPK0	AKO0HPK8	AKO3HPK8	AKO5HPK8	AKO7HPK8
Soybean meal[1]	203.9	203.9	203.9	203.9	189.8	189.8	189.8	189.8	189.8
Wheat flour[2]	200.0	200.0	200.0	200.0	220.0	220.0	220.0	220.0	220.0
Salmon meal[3]	180.0	180.0	180.0	180.0	298.0	220.0	220.0	220.0	220.0
Krill meal, high protein[4]	–	–	–	–	–	80.0	80.0	80.0	80.0
Wheat gluten meal[5]	100.0	100.0	100.0	100.0	50.0	50.0	50.0	50.0	50.0
Soy protein concentrate[6]	80.0	80.0	80.0	80.0	70.0	70.0	70.0	70.0	70.0
Poultry viscera and bone meal[7]	44.5	44.5	44.5	44.5	–	–	–	–	–
Soybean oil	44.6	35.6	17.6	–	24.8	40.0	35.6	18.0	0.6
Cassava starch	29.9	29.7	29.4	28.6	16.7	23.8	23.9	23.8	23.7
Calcium carbonate	20.2	20.2	20.2	20.2	23.3	19.0	19.0	19.0	19.0
Salmon oil	20.0	20.0	20.0	20.0	4.00	19.2	0.4	0.4	–
Astaxanthin krill oil[8]	–	10.0	30.0	50.0	–	–	30.0	50.0	70.0
Soy lecithin oil	14.6	13.7	11.8	9.9	3.5	6.6	3.8	1.9	–
Monosodium phosphate	15.8	16.0	16.2	16.4	10.0	10.0	10.0	10.0	10.0
Potassium chloride	13.4	13.4	13.4	13.4	13.1	14.2	14.3	14.3	14.3
Magnesium sulphate	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin-mineral premix[9]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Synthetic binder[9]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
L-Lysine[9]	3.6	3.6	3.6	3.6	0.3	0.1	0.1	0.1	0.1
DL-methionine[9]	1.5	1.5	1.5	1.5	1.2	0.9	0.9	0.9	0.9
L-Threonine[9]	1.3	1.3	1.3	1.3	1.2	1.0	1.0	1.0	1.0
L-Tryptophan[9]	–	–	–	–	10.0	3.7	3.7	3.7	3.7
Vitamin C[9]	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Chelated copper[9]	0.1	0.1	0.1	0.1	–	0.1	0.1	0.1	0.1
Chelated manganese[9]	0.03	0.03	0.03	0.03	0.04	0.05	0.05	0.05	0.05
Cholesterol[9]	–	–	–	–	1.2	5.0	0.9	0.5	0.2
Salt, coarse	–	–	–	–	0.3	0.1	0.1	0.1	0.1
Proximate composition (g/kg of the diet, as-is basis)									
Dry matter	897.1	881.5	893.8	898.8	909.8	906.9	909.7	904.5	911.9
Crude protein	409.2	406.4	406.0	409.6	417.5	408.9	413.7	411.3	413.3
Ether extract	80.7	75.1	97.1	101.0	77.0	78.6	80.3	89.4	97.6

Ingredients	Outdoor tanks				Indoor tanks				
	Astaxanthin krill oil (AKO)				Astaxanthin krill oil (AKO) & high-protein krill meal (HPK)				
	AKO0	AKO1	AKO3	AKO5	AKO0HPK0	AKO0HPK8	AKO3HPK8	AKO5HPK8	AKO7HPK8
Crude fibre	18.4	20.9	27.8	21.4	23.3	23.5	26.5	23.1	25.3
Crude ash	99.3	98.0	99.2	116.1	107.1	115.9	110.6	119.3	109.0
NFE[9]	289.5	281.1	263.7	250.7	284.9	280.0	278.6	261.4	266.7
GE (MJ/kg)[9]	197.1	195.6	200.3	198.6	195.8	194.1	195.7	195.9	199.7

[1]Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil). 103.0 g/kg moisture, 473.8 g/kg crude protein (CP), 22k8 g/kg ether extract (EE), 59.9 g/kg crude fiber (CF), 60.5 g/kg ash, 6.1 g/kg methionine (Met), 28.8 g/kg lysine (Lys), 12.8 g/kg M + C (methionine + cysteine).

[2]130.9 g/kg moisture, 112.4 g/kg CP, 5.3 g/kg EE, 1.9 g/kg CF, 6.8 g/kg ash, 1.7 g/kg Met, 2.5 g/kg Lys, 4.2 g/kg M + C.

[3]Pesquera Pacific-Star (Puerto Montt, Chile). 108.9 g/kg moisture, 644.4 g/kg CP, 87.1 g/kg EE, 2.1 g/kg CF, 161.2 g/kg ash, 18.7 g/kg Met, 49.7 g/kg Lys, 27.0 g/kg M + C.

[4]Qrill™ High Protein meal (Aker Biomarine Antarctic AS, Lysaker, Norway). 70.0 g/kg moisture, 700.0 g/kg CP, 70.0 g/kg EE, 120.0 g/kg ash, 21.0 g/kg Met, 50.0 g/kg Lys, 44.0 g/kg M + C.

[5]Amytex 100. Tereos Syral S.A.S. (Marckolsheim, France). 67.5 g/kg moisture, 796.8 g/kg CP, 24.4 g/kg EE, 4.1 g/kg CF, 18.7 g/kg ash, 11.6 g/kg Met, 13.5 g/kg Lys, 26.8 g/kg M + C.

[6]XSoy600 (Sementes Selecta S.A., Araguari, Brazil). 72.3 g/kg moisture, 616.4 g/kg CP, 23.5 g/kg EE, 33.1 g/kg CF, 68.0 g/kg ash, 8.0 g/kg Met, 37.6 g/kg Lys, 16.2 g/kg M + C.

[7]BRF S.A. (São Cristovão Capinzal, Brazil). 40.8 g/kg moisture, 697.9 g/kg CP, 136.6 g/kg EE, 14.0 g/kg CF, 84.8 g/kg ash, 14.9 g/kg Met, 43.6 g/kg Lys, 23.5 g/kg M + C.

[8]QRILL™ AstaOmega Oil (Aker Biomarine Antarctic AS, Lysaker, Norway). See Table 1 for composition.

[9]Vaccinar Industria e Comercio Ltda. (Pinhais, Brazil). Guarantee levels per kg of product: vitamin A, 1,200,000 IU; vit. D3, 200,000 IU; vit. E, 60,000 mg; vit. K3, 1,000 mg; vit. B1, 2,400 mg; vit. B2, 2,400 mg; vit. B6, 6,000 mg; vit. B12, 4 mg; nicotinic acid, 10,000 mg; pantothenic acid, 5,200 mg; biotin, 20 mg; folic acid, 400 mg; vit. 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; Cr, 60 mg.

[10]Nutri-Bind Aqua Veg Dry, Nutri-Ad International NV (Dendermonde, Belgium). Synthetic binder consisting of calcium lignosulfonate (940 g/kg.) and guar gum (60 g/kg).

[11]Biolys® (Evonik Nutrition and Care GmbH, Hanau, Germany). L-Lysine 546 g/kg.

[12]MetAMINO® (Evonik Nutrition and Care GmbH, Hanau, Germany). DL-Methionine 990 g/kg.

[13]ThreAMINO® (Evonik Nutrition and Care GmbH, Hanau, Germany). L-Threonine, 985 g/kg.

[14]TrypAMINO® (Evonik Nutrition and Care GmbH, Hanau, Germany). L- Tryptophan, 980 g/kg.

[15]Rovimix® Stay C® 35. Minimum of 350 g/kg of phosphorylated vitamin C activity (DSM Nutritional Products AG, Schweiz, Switzerland).

[16]Mintrex® Cu (Novus International, Inc., St. Charles, USA). 458 g/kg. CP, 780 g/kg HMTB content (784 g/kg methionine activity), 150,000 ppm copper.

[17]Mintrex® Mn (Novus International, Inc., St. Charles, USA). 447 g/kg CP, 760 g/kg HMTB content (764 g/kg. methionine activity), 130,000 ppm manganese.

[18]Cholesterol SF (Dishman Netherlands B.V., Veenendaal, Netherlands). Minimum of 910 g/kg of active cholesterol.

[19]Nitrogen-free extract. Calculated by difference [dry matter − (CP + EE + CF + ash)].

[20]Gross energy (GE, MJ/kg) given on a dry matter (DM) basis. Calculated as GE = (4,143 + (560 × EE [DM]) + (150 × crude protein [DM]) − (440 × crude ash [DM])) × 0.0041868. Source: Ewan (1989).

TABLE 3 Amino acid composition (g/kg, as-is basis) of experimental diets

	Outdoor tanks				Indoor tanks				
	Astaxanthin krill oil (AKO)				Astaxanthin krill oil (AKO) & high-protein krill meal (HPK)				
Amino Acids	AKO0	AKO1	AKO3	AKO5	AKO0HPK0	AKO0HPK8	AKO3HPK8	AKO5HPK8	AKO7HPK8
Essential Amino Acids (EAA)									
Arginine	23.3	23.2	23.1	23.4	24.5	23.8	24.4	24.1	24.2
Histidine	8.6	8.5	8.0	8.3	9.1	8.7	9.2	9.0	9.2
Isoleucine	15.3	15.5	15.1	15.6	15.1	15.7	15.9	16.0	16.0
Leucine	28.1	28.3	27.6	28.4	27.8	28.1	28.6	28.7	28.9
Lysine	20.8	20.7	20.3	20.8	22.8	21.6	22.0	21.8	22.2
Methionine	8.2	8.1	8.2	8.0	8.8	8.9	8.7	8.4	8.9
Met + Cys[1]	14.6	14.5	14.2	14.3	13.4	14.6	14.4	14.0	14.5
Phenylalanine	18.2	18.3	17.9	18.3	17.5	17.6	18.0	17.9	18.0
Threonine	15.4	15.5	15.4	15.4	16.6	15.9	16.5	16.3	16.4
Tyrosine	12.2	12.1	12.2	12.2	12.1	12.3	12.6	12.5	12.5
Valine	17.7	17.8	17.4	17.8	17.6	17.9	18.1	18.2	18.1
Non-Essential Amino Acids (NEAA)									
Alanine	17.9	17.9	17.8	17.8	19.8	19.0	19.4	19.3	19.5
Aspartic acid	32.6	34.1	33.2	33.8	36.0	35.0	36.3	35.9	36.0
Cysteine	6.4	6.4	6.0	6.3	4.6	5.7	5.7	5.6	5.6
Glycine	22.1	22.3	22.2	22.3	25.5	23.1	23.3	23.2	23.3
Glutamic acid	82.3	82.5	82.0	83.2	73.1	71.3	72.5	71.7	72.1
Proline	27.5	27.6	27.6	27.8	25.1	24.4	24.7	24.7	24.7
Serine	18.9	18.8	19.0	18.7	18.9	18.5	19.2	18.7	19.0
Taurine	1.9	1.9	1.8	2.0	3.0	3.0	2.7	3.0	3.0
Sum EAA[2]	167.8	168.0	165.2	168.2	171.9	170.5	174.0	172.9	174.4
Sum NEAA	209.6	211.5	209.6	211.9	206.0	200.0	203.8	202.1	203.2
EAA + NEAA	377.4	379.5	374.8	380.1	377.9	370.5	377.8	375.0	377.6

[1]TSAA, total sulphur amino acids.

[2]Tryptophan not analysed.

hatcheries, Aquatec Aquacultura Ltda. (Canguaretama, Brazil) and Samaria Unidade de Pós-Larvas Ltda. (Nísia Floresta, Brazil) used in the outdoor and indoor tanks, respectively. In both cases, shrimp originated from non-specific pathogen-free broodstock.

Two batches of 288,000 and 60,000 shrimp as PL10 were transported with 667 animals L^{-1} (10,000 PLs/bag) in double-plastic bags individually housed in cardboard boxes lined internally with Styrofoam. At arrival to the laboratory, shrimp were immediately acclimated to water temperature by placing the plastic bags into tanks with seawater at ambient temperature.

The 1st batch of PLs with 3.6 ± 0.3 mg BW were stocked in the outdoor tanks under 2,371 ± 20 PLs m^{-3} at a total of 3,556 ± 31 animals per tank ranging between 3,483 to 3,639 shrimp $tank^{-1}$. The 2nd batch with 2.5 ± 0.3 mg BW were stocked in the indoor tanks under 2,504 ± 58 PLs m^{-3} at a total of 1,252 ± 29 animals per tank (1,201–1,299 shrimp $tank^{-1}$).

Shrimp were counted using a portable smart device for rapid inventory assessment (XperCount2, XpertSea, Québec, Canada). After shrimp acclimation, each bag was opened for releasing PLs into a bucket containing clean seawater. PLs were captured with a small bag net and transferred to the XperCount2 bucket, which was set at a total quantity of near 1,000 shrimp requiring from three to four counting per rearing tank. After each counting, total PL biomass was determined by first removing excess water and then weighing shrimp to a 0.01-g resolution using an electronic scale. Subsequently, animals were transferred to their respective rearing tank. All rearing procedures were performed in compliance with relevant laws and institutional guidelines, including those related to animal welfare.

2.4 | Feed and system management

Diets were delivered in rearing tanks daily, including Sundays. In the outdoor system, shrimp were fed 20 times within 24-hour periods using an automatic feeder (Nunes et al., 2019). Daily meals were

TABLE 4 Fatty acid profile (g/kg of total dietary fatty acid, dry matter basis, DM) of experimental diets

	Outdoor tanks				Indoor tanks				
	Astaxanthin krill oil (AKO)				Astaxanthin krill oil (AKO) & high-protein krill meal (HPK)				
Fatty acids	AKO0	AKO1	AKO3	AKO5	AKO0HPK0	AKO0HPK8	AKO3HPK8	AKO5HPK8	AKO7HPK8
12:0	–	–	0.1	0.2	–	–	0.2	0.2	0.3
14:0	0.8	2.3	7.3	12.0	1.6	1.7	6.6	11.9	16.7
15:0	0.1	0.1	0.2	0.3	0.1	0.1	0.2	0.2	0.3
16:0	12.9	12.6	19.5	22.6	11.9	12.8	15.4	19.6	24.0
17:0	0.1	0.1	–	–	0.2	0.1	–	–	–
18:0	3.5	3.1	3.7	3.6	3.1	3.3	2.7	2.7	2.7
20:0	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1
21:0	0.1	0.3	0.9	1.3	0.3	0.3	0.8	1.3	1.8
22:0	0.2	0.2	0.3	0.4	0.3	0.3	0.2	0.2	0.3
24:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
14:1	–	–	0.1	0.1	–	–	0.1	0.1	0.2
17:1	0.1	0.1	0.4	0.7	0.1	0.1	0.4	0.8	1.0
16:1n-7	1.3	2.4	5.7	8.8	2.1	1.8	4.5	8.0	11.5
18:1n-9t	–	–	–	0.1	–	–	–	0.1	0.1
18:1n-9	22.4	21.6	25.7	25.1	22.9	20.4	17.5	18.5	20.0
20:1n-9	0.7	0.7	1.1	1.3	1.0	0.8	0.7	1.0	1.3
22:1n-9	0.1	0.1	0.1	0.1	0.1	0.1	–	0.1	0.1
24:1n-9	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
18:2n-6	38.2	32.8	31.6	22.4	28.1	33.4	27.7	21.0	11.6
20:2n-6	0.2	0.2	0.3	0.3	0.4	0.2	0.1	0.1	0.2
18:3n-6	–	–	0.1	0.2	–	–	0.1	–	0.2
20:3n-6	4.7	4.0	4.0	3.2	4.5	4.3	3.3	2.8	1.8
18:3n-3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	–
20:3n-3	0.4	0.5	0.6	0.7	0.7	0.4	0.3	0.4	0.5
20:4n-6	0.1	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.4
20:5n-3	1.2	1.7	3.5	4.9	2.5	2.9	4.2	6.1	7.9
22:6n-3	1.9	1.9	2.7	3.1	3.6	2.9	2.6	3.2	3.8
n-3[1]	8.2	8.1	10.7	11.9	11.3	10.5	10.4	12.5	14.0
n-6[2]	38.7	33.2	32.2	23.4	28.8	33.9	28.3	21.4	12.5
n-9[3]	23.3	22.5	27.2	26.9	24.2	21.4	18.2	19.8	21.6
SFA[4]	18.1	18.9	32.3	40.8	17.9	19.0	26.4	36.4	46.4
MUFA[5]	24.7	25.0	33.5	36.5	26.4	23.3	23.3	28.6	34.3
PUFA[6]	38.2	32.8	31.7	22.6	28.1	33.4	27.8	21.0	11.8
HUFA[7]	3.2	3.7	6.3	8.3	6.3	5.8	7.0	9.5	12.2

[1]n-3, 18:3n-3, 20:3n-3, 20:5n-3, 22:6n-3.

[2]n-6, 18:2n-6*t*, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6.

[3]n-9, 18:1n-9*t*, 18:1n-9, 20:1n-9, 22:1n-9, 24:1n-9.

[4]SFA, saturated fatty acids, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0.

[5]MUFA, monounsaturated fatty acids, 14:1, 15:1, 16:1, 18:1, 20:1, 22:1, 24:1.

[6]PUFA, polyunsaturated fatty acids, 18:2n-6, 18:3n-6.

[7]HUFA, highly unsaturated fatty acids, 20:4n-6, 20:5n-3, 22:6n-3.

placed in the feeding device between 07:00 and 07:30 a.m. For an increased control in the indoor tanks, diets were delivered exclusively in feeding trays nine times during the day, at 07:00, 08:00, 09:00, 10:00 and 11:00 a.m., and at 01:00, 02:00, 03:00 and 04:00 p.m. in the afternoon. Trays measured 14.3 cm in diameter and borders were 3.5 cm in height. Trays were installed in the middle of each tank

bottom at a density of one unit per tank. In both studies, meals were adjusted on a daily basis for each rearing tank assuming an estimated daily drop in shrimp survival and daily increase in shrimp BW across all diets.

Shrimp were first allowed to acclimate for 3 days prior to being fed on the experimental diets. During the acclimation period, 20% of the daily meal was composed of decapsulated Artemia cysts (Vitellus, Bern Aqua NV, Olen, Belgium) and the remainder a commercial nursery diet (Epac Black XL, INVE Thailand Ltd., Phichit, Thailand) containing a minimum of 450 g/kg CP and 70 g/kg lipid. Starting on the 10th day of rearing and then on a weekly basis, shrimp were sampled and weighed using a 0.001-g precision scale.

During weighing, shrimp were first collected, blotted dry with absorbent paper, counted individually and then weighed in bulk. Ten subsamples of five to ten shrimp each per tank were weighed to determine their mean BW and then returned to their respective tank. Until the next weight check, meals increased according to the mean daily weight gain achieved in the previous week in each tank, maintaining a consistent daily drop in survival.

Shrimp were fed different particle sizes during the nursery period (Figure 1). In the outdoor tanks, four particle sizes were used (<250–300, 425, and 600–850 μm) compared with six in the indoor tanks (<250, 250, 300, 425, 600 and 850 μm). The shift in particle size during nursery was carried out by visually analysing the ability of PLs to capture larger diet particles using a 1-L Becker glass stocked with 20 PLs. The new particle size was adopted when the number of successful attempts to capture (catch or grasp) the larger diet particle in the pre-oral cavity exceeded 70% (Nunes & Parsons, 1998). In order to adapt PLs to a new diet particle in the rearing tanks, the previous particle was mixed with the new one at 75%, 50%, 25% over a 3-day transition period.

2.5 | Growth performance

For shrimp harvest, water from each tank was drained slowly while a batch of 100 and 200 shrimp (outdoor and indoor systems, respectively) per rearing tank was captured and weighed individually to a

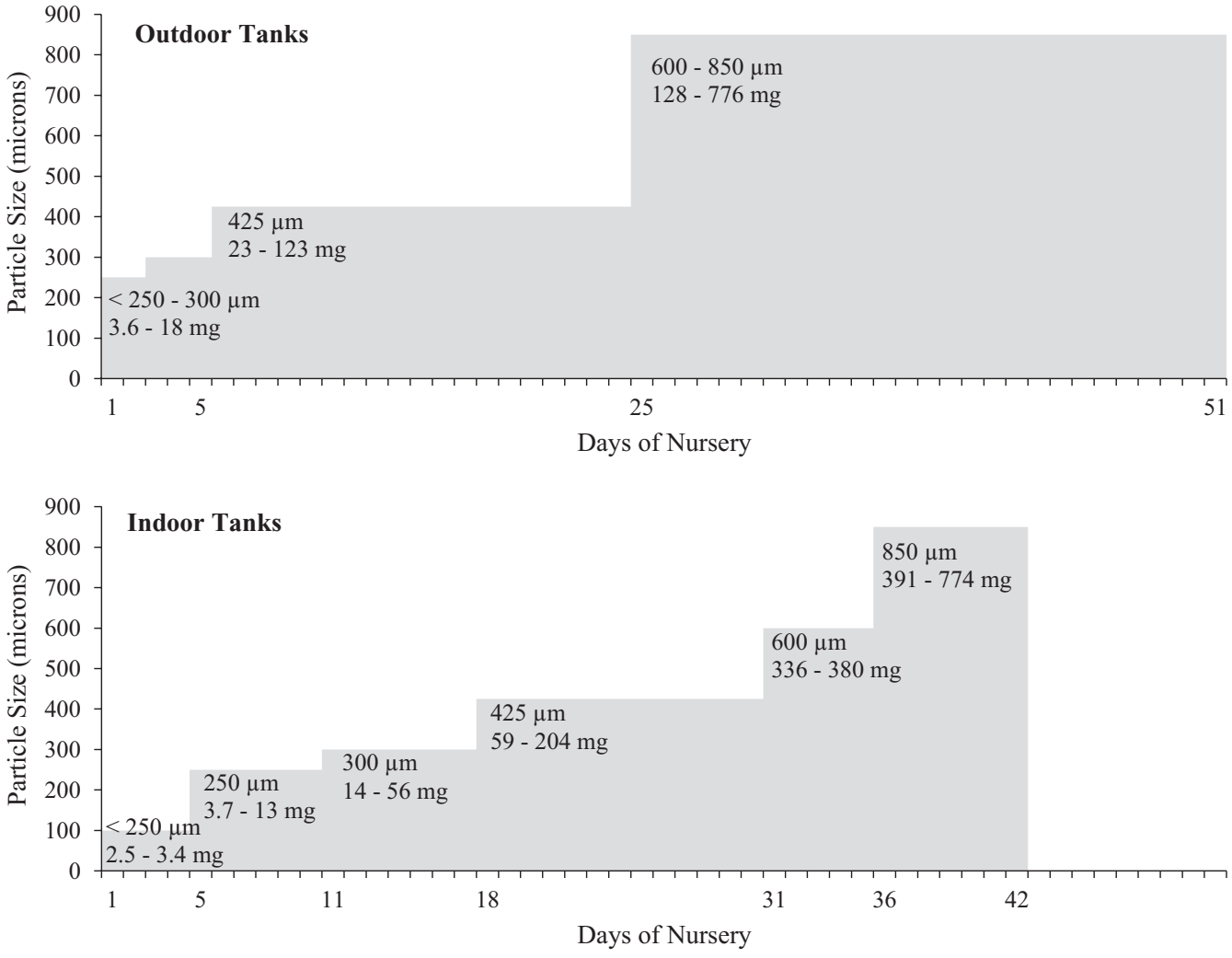

FIGURE 1 Transition of diet particle size (μm) as a function of shrimp body weight (mg) and day of nursery. Day 1 refers to a post-larva 10 (PL10)

0.001-g precision. Shrimp were first weighed wet in bulk and then blotted dry for individual weighing. These data were subsequently used to determine water content in the sample to estimate shrimp final survival. Finally, the tank was completely drained, and the remainder shrimp captured and weighed in bulk. The final shrimp population was estimated by dividing the total shrimp biomass (wet basis converted into blotted-dry basis) in each tank by the mean shrimp BW at harvest.

Shrimp final survival (S, %) was calculated by the equation: S = (POPf/POPi)×100, where POPi = number of shrimp at stocking, and POPf = number of shrimp at harvest. Daily weight gain (DWG, mg/day) was determined by the formula: WWG = [(Wf − Wi) ÷ *t*], in which Wi = shrimp wet body weight (BW, mg) at stocking, Wf = shrimp BW (mg) at harvest and *t* = number of days in culture. Gained shrimp yield (YIE, g of shrimp biomass gained m^{-3}) was determined as YIE = (BIOf − BIOi)/tank volume (m^3), where BIOi = initial shrimp biomass per tank (g) and BIOf = final shrimp biomass per tank (g). FCR was calculated by dividing the total amount of feed delivered (g, as-fed basis) during culture by the wet shrimp biomass gained (g) in each tank. Apparent feed intake (AFI, g $shrimp^{-1}$) was determined by dividing the total amount of feed delivered by the number of stocked shrimp.

2.6 | Stress test

In the outdoor tanks, harvested shrimp were evaluated in regards to their resistance to an abrupt change in water temperature and salinity. The temperature stress test was carried out using four round plastic buckets of 50 L each equipped with a digital thermometer and continuous aeration provided by an aeration stone. First, cold seawater was mixed with seawater under ambient temperature in order to achieve a mean temperature of 19.2 ± 0.9°C (*n* = 96). Ten shrimp were randomly collected from each dietary treatment and transferred to the bucket. Shrimp mortality was recorded after 10, 20 and 30 min. of exposure. Five replicate observations were carried out using different shrimp.

For the salinity stress test, 10 shrimp from each treatment were collected and transferred to a fibreglass tank split into four sections each with an individual volume of 6 L. Shrimp were simultaneously subjected to a drop in salinity from 39 to 0 g/L for 0, 10, 20 and 30 min. when shrimp mortality was determined. Three replicate observations were carried out.

2.7 | Statistical analyses

Statistical analyses were performed with the Statistical Package for Social Sciences, package 23 (IBM® SPSS® Statistics). The effect of dietary inclusion of AKO over shrimp performance and survival to osmotic and thermal stress was analysed through one-way ANOVA. Similarly, differences between water salinity, pH, temperature, TAN, nitrite and nitrate between dietary treatments and culture periods were analysed by one-way ANOVA. When significant differences were detected, they were compared two-by-two with the Tukey HSD test. Homogeneity of variance was examined for all data by using Bartlett-Box F and Cochran's C tests. Kurtosis and skewness and their standard error (i.e. *SEM* kurtosis and *SEM* skewness) were applied to the data as measures of asymmetry and tests of normality.

3 | RESULTS

In the outdoor and indoor tank systems, water pH, temperature and salinity remained within levels considered adequate for the farming of whiteleg shrimp. These parameters were unaffected by dietary treatment ($p > .05$). In the outdoor system, mean water salinity reached 35 ± 1 g/L (*n* = 1,200) slightly higher than the 32 ± 2 g/L (*n* = 1,200) recorded in the indoor tanks. In both experiments, there was a trend towards higher salinity as culture progressed since water was exchanged at a minimum. Temperature remained high with means ranging from 27.9 ± 0.3 (*n* = 1,040) to 28.4 ± 0.4°C (*n* = 1,200) in outdoor and indoor tanks, respectively. pH was stable with means of 7.7 ± 0.2 (*n* = 1,200, outdoor tanks) and 8.2 ± 0.3 (*n* = 1,200, indoor tanks), respectively.

Concentration of nitrogenous compounds did not vary significantly between dietary treatments ($p > .05$). In the outdoor tanks, TAN, NO_2-N and NO_3-N reached 0.52 ± 0.85, 2.15 ± 1.24 and 1.83 ± 0.80 mg/L, respectively. However, there were significant variations throughout the culture period. TAN remained within safe levels, below 1.5 mg/L. It progressively reduced during culture combined with the accumulation of both NO_2-N and NO_3-N.

In the outdoor tanks, no statistical effect from the inclusion of AKO was observed on final shrimp survival (84.0 ± 16.8%), gained yield (1,568 ± 181 g/m^3), daily growth (14.2 ± 2.8 mg/day), apparent feed intake (AFI, 1.12 ± 0.07 g of feed $shrimp^{-1}$) and feed conversion ratio (FCR, 1.70 ± 0.16, Table 5). However, statistical differences were detected in final shrimp BW between dietary treatments ($p < .0001$, Figure 2). A similar result was achieved for shrimp raised in the indoor tanks. There was no significant effect from the dietary inclusion of AKO and/or HPK over shrimp performance ($p > .05$), except in AFI and final BW. Mean final survival (91.8 ± 14.2%) was higher than in the outdoor tanks, but it did not differ statistically between dietary treatments. Similarly, gained yield (1,611 ± 181 g/m^3), daily growth (14.2 ± 1.8 mg/m^3) and FCR (0.89 ± 0.09) were not significantly affected by dietary treatment. However, there was a significant effect of AKO over AFI, starting at 30 g/kg dietary inclusion (AKO3HPK8). AFI increased from 0.55 ± 0.02 g of feed $shrimp^{-1}$ when fed AKO0HPK0 to 0.58 ± 0.03 g of feed $shrimp^{-1}$ with diet AKO3HPK8. No statistical difference in AFI was observed between the control diet (AKO0HPK0) and the positive control diet without AKO containing 80 g/kg of HPK (AKO0HPK8).

Shrimp BW started to differentiate between dietary treatments early during nursery (Figure 2), that is after 10 days of rearing in both the outdoor and indoor tanks. In outdoor tanks, the dietary inclusion of 50 g/kg AKO (AKO5) significantly enhanced final shrimp BW (775 ± 140 mg) in comparison to all other diets. A dietary

TABLE 5 Growth performance (mean ± *SD*) of postlarval *L. vannamei* reared for 52 and 41 days in outdoor in indoor tanks and fed with diets containing AKO and/or HPK

Growth parameters	Diets/Astaxanthin krill oil (AKO)					
Outdoor tanks	**AKO0**	**AKO1**	**AKO3**	**AKO5**	**Mean ± *SD***	***p* ANOVA**
Initial body weight (mg)	3.7 ± 0.4	3.6 ± 0.3	3.6 ± 0.3	3.6 ± 0.3	3.6 ± 0.3	.859
Survival (%)	87.2 ± 15.8	86.3 ± 20.2	85.5 ± 14.0	76.8 ± 17.0	84.0 ± 16.8	.534
Gained yield (g/m^3)	1,612 ± 157	1,534 ± 193	1,595 ± 175	1,534 ± 213	1,568 ± 181	.717
Growth (mg/day)	13.9 ± 3.1	13.3 ± 2.1	14.4 ± 3.2	15.1 ± 2.7	14.2 ± 2.8	.548
AFI[a]	1.11 ± 0.08	1.12 ± 0.08	1.12 ± 0.08	1.13 ± 0.06	1.12 ± 0.07	.971
FCR[b]	1.63 ± 0.11	1.73 ± 0.15	1.66 ± 0.12	1.76 ± 0.22	1.70 ± 0.16	.292
	Diets/Astaxanthin krill oil (AKO) & high-protein krill meal (HPK)					
Indoor Tanks	**AKO0HPK0**	**AKO0HPK8**	**AKO3HPK8**	**AKO5HPK8**	**AKO7HPK8**	**Mean ± *SD***	***p* ANOVA**
Initial body weight (mg)	2.3 ± 0.4	2.5 ± 0.3	2.5 ± 0.3	2.6 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	.204
Survival (%)	89.2 ± 10.7	91.6 ± 10.4	89.6 ± 13.9	92.3 ± 13.5	97.1 ± 22.7	91.8 ± 14.2	.808
Gained yield (g/m^3)	1,513 ± 88	1,593 ± 286	1,636 ± 91	1,627 ± 92	1,708 ± 252	1,611 ± 181	.234
Growth (mg/day)	13.5 ± 1.5	13.8 ± 1.3	14.9 ± 1.8	14.5 ± 1.9	14.5 ± 2.5	14.2 ± 1.8	.450
AFI (g)[1]	0.55 ± 0.02a	0.56 ± 0.02ab	0.58 ± 0.03bc	0.58 ± 0.03abc	0.59 ± 0.02c	–	.019
FCR[2]	0.92 ± 0.06	0.90 ± 0.13	0.88 ± 0.07	0.88 ± 0.04	0.86 ± 0.13	0.89 ± 0.09	.723

Note: Common letters indicate non-statistically significant differences between days of rearing according to the Tukey's HSD test at the $\alpha = 0.05$ level.

[1]Apparent feed intake is the amount of feed delivered divided by the number of stocked shrimp.

[2]Feed conversion ratio.

inclusion of 30 g/kg AKO (AKO3) also resulted in an increased BW (739 ± 165 mg) compared with 10 g/kg (AKO1, 683 ± 105 mg), but not to the control (AKO0, 711 ± 157 mg). A similar growth response was detected in indoor tanks. Shrimp fed diets containing AKO significantly enhanced final BW starting at 30 g/kg dietary inclusion (AKO3HPK8, 629 ± 76 mg), but not beyond this level. Shrimp fed AKO5HPK8 and AKO7HPK8 (613 ± 81 and 612 ± 105 mg, respectively) did not improve BW compared to AKO3HPK8 or to the diet with HPK only (AKO0HPK8, 583 ± 55 mg). There was no positive effect over shrimp final BW as a result of the inclusion of 80 g/kg HPK since it did not differ from shrimp fed the control diet (AKO0HPK0, 567 ± 65 mg).

AKO had a significant effect on survival when shrimp were subjected to a severe drop in both temperature or salinity (Figure 3). When water salinity dropped from 39 g/L to 0, shrimp survival reduced with a longer time of exposure. At 30 min. of exposure, there was a clear trend towards higher survival with a progressive increase in the dietary inclusion of AKO. A minimum of 1 g/kg AKO (AKO1, 80.0 ± 10.00%) was sufficient in promoting an enhanced survival compared to the control diet (66.7 ± 15.28%). However, at 50 g/kg (AKO5), no shrimp mortality was recorded after 30 min of exposure. Significant differences in shrimp survival between dietary treatments were observed after 20 min of exposure to a drop in water temperature. At 30 min, cumulative survival for shrimp fed AKO0 was the lowest compared to all other dietary treatments fed AKO.

4 | DISCUSSION

In our work, there were marked differences in PL survival and FCR under the two rearing tank systems. PLs nursed in indoor tanks achieved a higher survival with lower FCR compared with animals reared in outdoor tanks. This suggests that the outdoor system imposed a greater level of stress to postlarval *L. vannamei*. In this system, there was less environmental control in regard to algae growth, sunlight and rainfall exposure, water salinity and temperature, and the concentration of nitrogenous compounds. Under the indoor system, weekly water exchange allowed the discharge of suspended and settled organic matter as opposed to the outdoor system which operated under minimum-water exchange. Previous studies have indicated that clear water may lead to a better growth performance in both postlarval and juvenile *L. vannamei* compared with a biofloc system. Esparza-Leal et al. (2015) evaluated the growth performance of postlarval *L. vannamei* stocked under 1,500, 3,000, 6,000 and 9,000 PLs m^{-3} in a clear-water versus biofloc system. After a 42-day nursery period, authors reported that PLs achieved a higher mean final BW (from 1.26–0.51 versus from 0.62–0.34 g at the lowest and highest stocking density, respectively) and a higher specific growth rate in a clear (9.7–11.8% day^{-1}) compared with a biofloc (8.6–10.1% day^{-1}) nursery system. Similarly, Ray et al. (2017) evaluated the growth performance of juvenile *L. vannamei* raised under clear-water RAS and biofloc systems. Shrimp weighing 0.42 g were stocked under 250

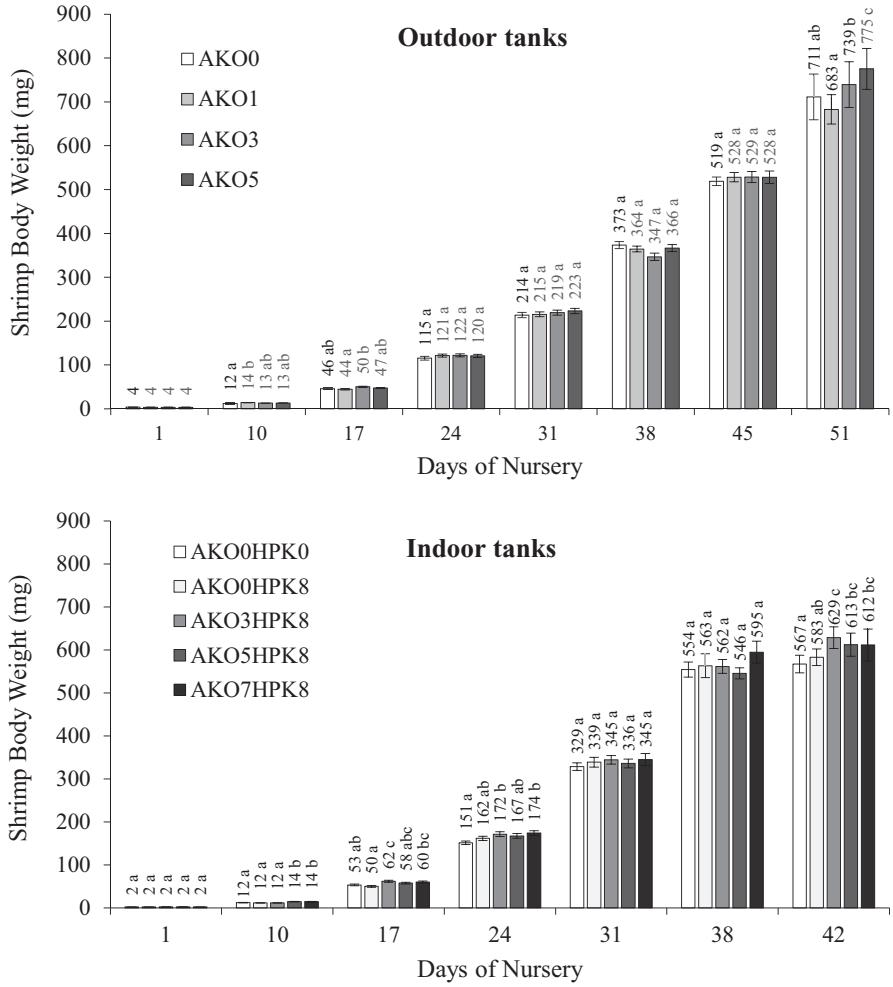

FIGURE 2 Progressive gains in mean shrimp body weight ± *SEM* (BW, mg) over the two experimental culture periods. Shrimp were fed with graded levels of dietary astaxanthin krill oil (AKO). Each column represents the mean BW of 50–100 shrimp weighed in batches of 10 animals. Common letters in each day of nursery indicate non-statistically significant difference according to Tukey's HSD test at the 0.05 level

animals m^{-3} and grown for 55 days. Authors found that shrimp mean harvest weight and biomass were significantly greater, and FCR was significantly lower in the clear-water treatment. There were no significant differences in survival.

Since the two experiments were carried out under different sunlight exposure conditions, this may have affected pH between the two trials. Different pH patterns and the build-up of NO_3-N during culture suggest that nitrification was taking place. TAN levels tended towards reaching zero concentration, while NO_2-N and NO_3-N were far from reaching levels outside the ideal range for *L. vannamei* (10 and 450 mg/L, respectively). In addition to different rearing conditions in water quality (pH, salinity, temperature, etc.), the two nurseries also differed in relation to the PL source, initial shrimp BW, feeding frequency, diet composition and their particle sizes. Diets were formulated to be more challenging in terms of nutrient content and digestibility for shrimp reared outdoors. In the indoor system, about 30% of diet composition was derived from marine proteins (salmon meal and/or HPK) compared with 18% in the outdoor tanks.

Regardless of the rearing system, the dietary supplementation of AKO resulted in an improvement of BW in postlarval *L. vannamei* in both rearing systems. In outdoor tanks, the inclusion of 50 g/kg AKO enhanced shrimp final BW. Comparatively, under a more controlled rearing condition, in indoor tanks, 30 g/kg AKO with 80 g/kg HPK was sufficient to deliver a significant improvement in final shrimp BW after 42 days of rearing.

Analysed proximate and AA composition remained relatively the same within each set of diets used in the outdoor and indoor tanks. Thus, the observed positive effect over shrimp growth performance was likely driven by a higher dietary supply of astaxanthin and the long-chain n-3 HUFA, EPA and DHA. AKO contains lower levels of linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids compared with the salmon oil and soybean used (NRC, 2011). Thus, the progressive replacement of these oil sources for AKO resulted in lower levels of short-chain n-3 and n-6 PUFA in the diets. In the 2nd set of diets, replacement of salmon oil and salmon meal for AKO and HPK resulted in dietary EPA ranging from 4.2–7.9 g/kg above the levels found in the negative control diet (2.5 g/kg, AKO0HPK0). However, dietary DHA was reduced, but the minimum recorded level (2.6 g/kg, diet AKO3HPK8) approached the value detected in diet AKO3 (2.7 g/kg) used in the outdoor system.

For the whiteleg shrimp, the long-chain HUFA (ARA, EPA and DHA) have a much higher nutritional value than the shorter-chain, LNA and LOA (González-Félix et al., 2003). The species does not appear to have a dietary requirement for LNA and LOA (González-Félix et al., 2003), so their levels were likely less critical for improving the growth performance of postlarval *L. vannamei*. On the other hand,

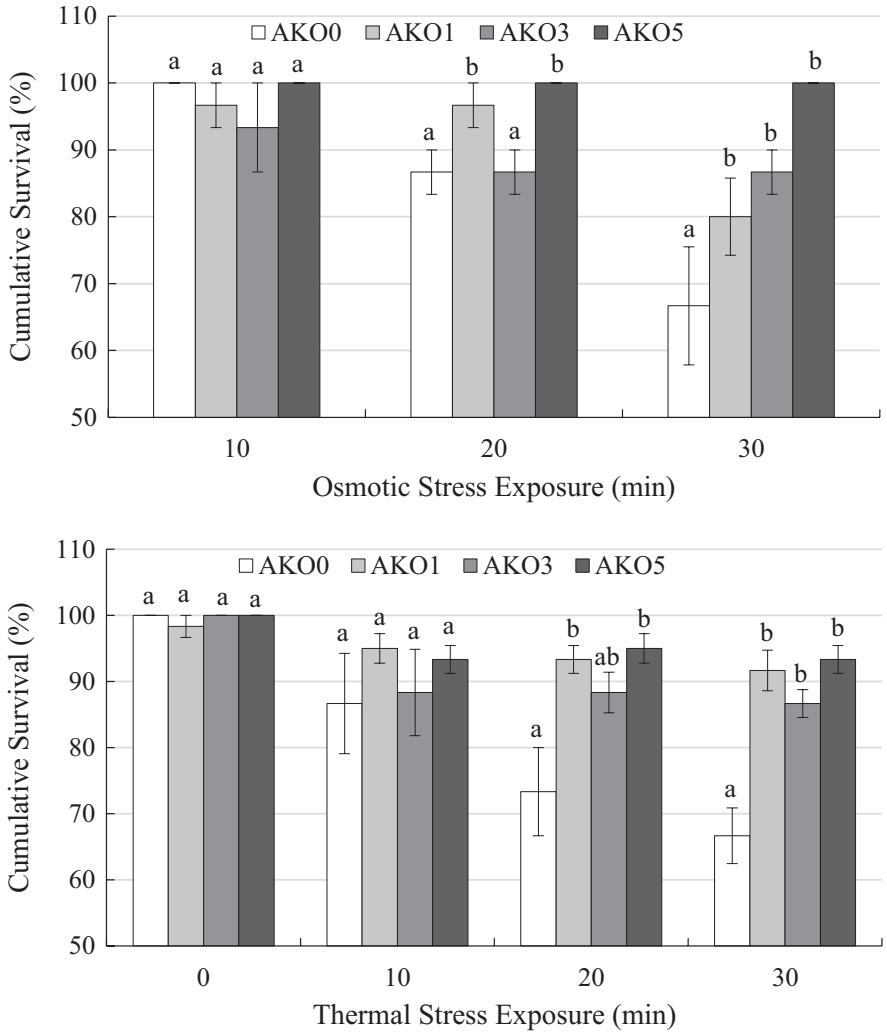

FIGURE 3 Mean (±*SEM*) cumulative shrimp survival (%) after sudden exposure to osmotic and thermal stress for 0, 10, 20, and 30 min. Common letters indicate non-statistically significant difference according to Tukey's HSD test at the 0.05 level

our diets have exceeded the recommended quantitative levels of n-3 HUFA for optimum growth of juvenile *L. vannamei* (0.4–4.7 g BW), which has been determined to be 5 g/kg of the diet (González-Félix et al., 2002). In both the outdoor and indoor culture systems, shrimp BW was maximized with a total dietary HUFA (sum of ARA, EPA and DHA) content of 8.3 and 7.0 g/kg, respectively. In comparison, the water salinity of 25 g/L in González-Félix et al.'s (2002) work was within the isosmotic point for *L. vannamei* (21.1–26.1 g/L; Castille & Lawrence, 1981; Gong et al., 2004; Jaffer et al., 2020). In our study, salinity reached higher concentrations (35 ± 1 outdoor and 32 ± 2 g/L indoor) which may have led to a higher demand for n-3 HUFA during hyper-osmoregulation. Shrimp will adapt to high salinity conditions by increasing their gill membrane composition with fatty acids containing high unsaturation indexes which provide a higher ion and water permeability (Souza et al., 2016). Under hypersalinity (>35 g/L), juvenile *L. vannamei* fed HUFA-enriched diets contain higher levels of EPA and DHA in their hepatopancreas and gills (Hurtado et al., 2007) and are able to achieve a better growth performance (Castro et al., 2018; Hurtado et al., 2007; Rufino et al., 2020).

A continuous 52-day exposure to diets containing at least 10 g/kg AKO also led to a higher survival when early juvenile shrimp (between 683 and 775 g BW) were exposed to a sudden and significant drop in water temperature and salinity after harvest. After a 30-min osmotic shock from 39 to 0 g/L salinity, no shrimp mortality was observed for shrimp that had been fed 50 g/kg AKO. While such a sudden drop in salinity is unlikely to occur during regular shrimp culture, this result suggests a greater resistance of shrimp to osmotic stress when fed AKO-supplemented diets. Various investigations have shown that astaxanthin improves shrimp resistance and growth performance. Liu et al. (2018) evaluated a synthetic (Carophyll® Pink 10%, DSM) and a natural source (*Haematococcus pluvialis* cell powder) of astaxanthin in diets for postlarval *L. vannamei* at 0, 50, 70, 90 and 140 ppm. Authors reported that shrimp fed the natural source of astaxanthin for 35 days achieved a higher growth performance and astaxanthin content compared with shrimp fed the synthetic astaxanthin. At 90 ppm, shrimp fed diets supplemented with the natural astaxanthin showed higher mRNA expression levels for the antioxidant enzymes (cMnSOD and GPx) and a lower cumulative mortality after a 72-hr challenge to *Vibrio parahaemolyticus*. Likewise, Darachai et al. (1998) observed increased survival in PLs of *Penaeus monodon* fed diets supplemented with astaxanthin. The authors also found that the use of natural carotenoid sources (*H. pluvialis*) was more effective in increasing shrimp resistance than synthetic sources. Chien and Jeng (1992) reported increased survival as a beneficial effect of

astaxanthin supplementation in *Marsupenaeus japonicus* diets. Pan et al. (2003) reported that a supplementation of 71.5 mg/kg astaxanthin for 56 days promoted greater survival (70 vs. 52%) and final BW (3.6 vs. 3.4 g) in juvenile *P. monodon* shrimp. Authors concluded that astaxanthin is a semi-essential nutrient for *P. monodon*, particularly under thermal and osmotic stress conditions, because of its antioxidant properties. Thus, in the present study, the antioxidant properties of astaxanthin may have supported a greater resistance to osmotic and thermal stress of whiteleg shrimp fed AKO-supplemented diets.

In the indoor tanks, a combination of AKO and HPK resulted in a higher AFI. Although AFI may be the response of higher shrimp growth rates, it also suggests that diets were more attractant and palatable to shrimp. Previous work has shown that krill meal is a powerful feed attractant and growth enhancer to juvenile *L. vannamei* (Nunes et al., 2019). Therefore, the higher observed growth performance may also be related to a greater attractiveness that diets with AKO and HPK conferred. As opposed to salmon oil, AKO is partially water soluble and this may assist in the leaching of low molecular weight stimulatory compounds that confer an increased feed attractiveness and palatability.

5 | CONCLUSIONS

Our findings have shown that the dietary inclusion of 50 g/kg AKO or 30 g/kg AKO when combined with 80 g/kg HPK to a diet fed to postlarval *L. vannamei* (2.5 and 3.6 mg initial BW) raised under high density result in an enhanced growth performance during 51 and 42-day nursery periods, respectively. Additionally, shrimp that had been previously fed a diet with 50 g/kg AKO showed no mortality after a 30-min exposure to acute osmotic stress. Shrimp fed with only 1 g/kg AKO also exhibited greater resistance to thermal stress. These findings may represent a significant advantage to the hyper-intensive nursery culture of whiteleg shrimp as they give the possibility to shorten the production cycle and reduce the risk of shrimp mortality after transfer to grow-out ponds. We have hypothesized that the enhancement in growth performance and resistance to environmental stress of postlarval *L. vannamei* may be related to the increased supply of dietary EPA, DHA and astaxanthin that the dietary supplementation of AKO and HPK provided. Further work should explore if the acquired beneficial effects during nursery can be sustained at later stages of shrimp growth.

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CONFLICT OF INTEREST

Aker Biomarine Antarctic AS donated the astaxanthin krill oil and the high protein krill meal analysed in this study. This did not alter, influence, or affect the development of the study, including study design, sampling, analysis of results, interpretation of data, or decision for publication. Authors declare no other conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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