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Fermented corn protein concentrate to replace fishmeal in practical diets for Pacific white shrimp *Litopenaeus vannamei*

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Abstract

The latest corn-based ingredient available in the market today is fermented corn protein concentrate (FCPC), which is expected to perform well in shrimp diets due to its nutritional profile and the probiotic properties of the product. The current study was conducted to evaluate the efficacy of FCPC as a replacement for fish meal (FM) in diets of Pacific white shrimps, *Litopenaeus vannamei*. Graded levels of FCPC (0, 4, 8, 11, 13 and 15 g/100 g) were used to replace fishmeal (16, 12, 8, 4, 2 and 0 g/100 g) in diet, which was evaluated over a 8-week growth trial (initial weight 0.17 ± 0.01 g; $n = 3$). At the conclusion, no significant differences were detected in growth, FCR, survival or haematological parameters of shrimp ($p > 0.05$). Results reveals the efficacy of FCPC to replace 100% fishmeal at an inclusion level as high as 15%, without compromising the performances of shrimp. Significant increase in total haemocyte count (THC) and astaxanthin level in shrimp in response to the inclusion level of FCPC was assumed to be due to the probiotic properties of fermented corn and due to the availability of bio-convertible carotenoids in FCPC, respectively.

KEYWORDS

astaxanthin, fermented corn, fish meal, growth, *Litopenaeus vannamei*, total haemocyte count

1 | INTRODUCTION

Fishmeal (FM) has traditionally been the main protein source used in aquaculture feed formulations due to its high protein content, balanced essential amino acid and fatty acids profiles, higher levels of digestible energy, vitamins and minerals contents, and higher palatability (Amaya et al., 2007a; Tacon et al., 2009). However, because of static supply, increasing demand, price and ethical issues, average dietary fish meal inclusion levels within compound feed for shrimp have been steadily declining (Jackson, 2012). As an alternative, wide varieties of plant-based dietary ingredients have been tested (Amaya et al., 2007a; NRC, 2011), including some of the co-products from corn milling industry (Yua et al., 2013; Zhou et al., 2014; Molina-Poveda et al. 2015; Rhodes et al., 2015; Guo et al., 2019).

Increased demand for ethanol as a fuel additive has resulted in dramatic growth in ethanol production from corn, by either wet

milling or dry-grind processing (Rausch & Belyea, 2006). In wet milling, the corn kernel is fractionated into different components, resulting in several co-products such as corn oil, corn gluten feed (CGF) and corn gluten meal (CGM) (Malumba et al., 2015; Rausch et al., 2003; Singh et al., 2006). Corn gluten feed (CGF) is the fibre-rich component removed in the wet milling process with low protein content (~20%) used mainly as a feed ingredient in the diets of cattle (Krehbiel et al., 1995; White & Johnson, 2003) and catfish (Hu et al., 2012; Li et al., 2011). CGF used as an inexpensive 'high-protein' carbohydrate source in low-to-moderate protein feeds. Corn gluten meal (CGM) is a high-protein (~60%), low-fibre fraction rich in vitamins B and E, utilized as a feed ingredient for poultry and fish feed (Navarro et al., 2016; Ramirez et al., 2008). CGM is known to contain no antinutritional factors (ANF), but low in amino acids such as arginine and lysine when compared with fishmeal (Regost et al., 1999). Amaya et al., (2007b) observed no significant differences in

performances of shrimp fed 4.8% of CGM, completely replacing FM protein in diets under semi-intensive production conditions in earthen ponds. However, in an outdoor pond trial, Forster et al., (2002), have observed a significant reduction in growth performance of shrimp fed a diet containing 15% CGM (with 0% FM) compared with a 11% fishmeal-based control diet. Molina-Poveda et al., (2015) also reported a significant drop in growth when shrimp fed a diet formulated with 8.6% or higher inclusion levels of CGM in a system devoid of endogenous food.

Increasing global competition and changes in demand, technological advances and novel findings through research are causing the ingredient manufacturing industry to embrace new processing technologies and new ingredients. Corn protein concentrate (CPC) is a newly manufactured corn protein source, which has a higher protein content (~75%) than that of CGM (Table 1). CPC is the dried protein fraction of the corn primarily originating from the endosperm after removal of the majority of non-protein components by enzymatic solubilization of the protein stream obtained from the wet milling process. Yu et al., (2013) demonstrated the efficacy of using 12% CPC as a complete replacement of dietary FM in the diet for Pacific white shrimp without compromising the growth, FCR and survival (Rhodes et al., 2014). These results from

pond trial were found to be inconsistent with the trial conducted in indoor re-circulatory system, which showed a significant negative impact of CPC in diet to the growth performance of shrimp (Zhou et al., 2014). The depressed growth exhibited by shrimp fed high inclusions of CGM and CPC was attributed to number of factors including reduced feed intake, protein and amino acid digestibility, and possible deficiencies in essential amino acids (specifically arginine and lysine) (Lemos et al., 2009; Yang et al., 2009; Molina-Poveda et al. 2015). However, CGM and CPC are often added as high protein ingredients and methionine supplements, due to their high methionine levels compared with other plant-based proteins (Davis & Duan, 2017).

The latest corn-based protein source currently available in the market for aquaculture feed formulation is fermented corn protein concentrate (FCPC). This product is predicted to be beneficial in improving growth and feed conversion in shrimps due to its remarkable nutritional profile (high protein, no ANF, low fibre, etc.) and is expected to boost the immune response of shrimp due to the probiotic effects of the product. Therefore, the current study was conducted to evaluate the efficacy of fermented corn protein concentrate (FCPC) as a replacement for fish meal in practical diets of Pacific white shrimps, *Litopenaeus vannamei*.

(g/100 g as is)	Corn ¹	CGF ¹	CGM ¹	CPC ²	FCPC ³	FM ²
Crude protein	8.24	17.39	58.25	78.07	69.10	64.75
Moisture	11.69	12.81	9.96	8.16	7.70	6.28
Crude fat	3.48	4.21	4.74	2.03	3.00	9.09
Crude fibre	1.98	7.08	0.70	0.87	1.00	0.66
Ash	1.3	5.14	1.46	1.03	6.00	19.77
Amino acids						
Alanine	0.6	1.28	4.33	6.42	5.90	4.01
Arginine	0.37	1.04	1.66	2.25	2.35	3.78
Aspartic acid	0.54	1.05	2.97	4.29	4.13	5.49
Cysteine	0.19	0.46	1.01	1.30	1.20	0.54
Glutamic acid	1.48	3.11	11.2	14.68	15.80	7.69
Glycine	0.31	0.79	1.28	1.95	2.00	4.97
Histidine	0.24	0.67	1.32	1.48	1.43	1.66
Isoleucine	0.28	0.66	2.23	2.96	2.67	2.56
Leucine	0.96	1.96	9.82	12.97	10.30	4.31
Lysine	0.25	0.63	0.93	1.14	2.95	4.89
Methionine	0.18	0.35	1.21	1.80	1.59	1.69
Phenylalanine	0.39	0.76	3.52	4.80	4.14	2.45
Proline	0.71	1.56	4.93	7.31	6.42	3.00
Serine	0.38	0.78	2.29	3.80	3.44	2.21
Threonine	0.28	0.74	1.81	2.48	2.29	2.50
Tryptophan	0.06	0.07	0.27	0.37	0.29	0.65
Tyrosine	0.26	0.58	2.86	4.24	3.44	1.92
Valine	0.38	1.01	2.42	3.23	3.31	2.97

TABLE 1 Proximate and amino acid composition (g/100 g as is) of corn, corn gluten feed (CGF), corn gluten meal (CGM), corn protein concentrate (CPC), fermented corn protein concentrate (FCPC) and fishmeal (FM)

Nutritional composition data sourced from, ¹National Research Council (2012), ²Guo et al., (2020) and ³Cargill, Minneapolis, MN, USA.

2 | MATERIALS AND METHODS

2.1 | Diet preparation

Six isonitrogenous and isolipidic (360 g/kg protein and 80 g/kg lipid) test diets were formulated using the FCPC sourced from Cargill, Minneapolis, MN, USA (Table 1). Graded levels of FCPC (0, 4, 8, 11, 13 and 15 g/100 g) were used to replace fishmeal (16, 12, 8, 4, 2 and 0 g/100 g) on an isonitrogenous basis, and the experimental diets were designated as FCPC0, FCPC4, FCPC8, FCPC11, FCPC13 and FCPC15, respectively (Table 2). In addition to FCPC and fishmeal, a fixed level of 46.3% of solvent-extracted soybean meal (Bunge Limited, Decatur, AL, USA) and 1% of corn protein concentrate (CPC Empyreal 75™, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA) was used as the dietary protein sources in diets.

The test diets were prepared in the feed laboratory of Auburn University, Auburn, AL, USA, using standard procedures for the laboratory production of shrimp feed. Briefly, preground dry ingredients and oil were weighted and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot water (~30% by weight) was then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (50°C) to a moisture content of <100 g/100 g. Dry pellets were crumbled and stored at 4°C until use. The test diets were analysed for proximate composition and amino acid profile at University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA; Tables 2 and 3).

2.2 | Experimental system

The growth trial was conducted in a semi-closed recirculation system consisting of 85-L fibreglass aquaria connected to a common reservoir tank (800-L) at E.W. Shell Fisheries Center at Auburn University, Auburn, Alabama, in compliance with the Auburn University animal care policy. Water quality was maintained by recirculation through an Aquadyne bead filter (0.2 m² media, 0.6 m × 1.1 m) and vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp. centrifugal pump. Mean water flow for an aquarium was 4 L/min with an average turnover of ~21 min/tank. Salt water was prepared by mixing manufactured sea salt (Crystal Sea® Marinemix, Baltimore, MD, USA) with dechlorinated freshwater and maintained at around 9.5 ppt during the trial. Dissolved oxygen was maintained near saturation using air stones in each culture tank and the sump tank using a common airline connected to a regenerative blower. During the feeding period, dissolved oxygen (DO), temperature and salinity were monitored twice daily (0830 and 1630) using an YSI 55 multi-parameter instrument (YSI, Yellow Springs, OH) and total ammonia nitrogen (TAN) and nitrite-N were measured twice per week using YSI 9500 photometer (YSI, Yellow Springs, OH). The pH of the water was measured twice weekly during the experimental period using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA), while alkalinity, hardness and nitrate level of water was measured twice per month using WaterLink Spin TouchFF photometer (LaMotte

Company, Chestertown, MD). During the growth trial, DO, temperature, salinity, pH, TAN, nitrite and alkalinity were maintained at 6.7 ± 0.4 mg/L, 28.4 ± 0.5 °C, 9.8 ± 0.5 ppt, 7.5 ± 0.5 , 0.3 ± 0.13 mg/L, 0.05 ± 0.01 mg/L and 98.0 ± 11.8 mg/L, respectively.

2.3 | Growth trial

Postlarval (PI) Pacific white shrimp (~0.003 g) for the experiment were obtained from American Mariculture, Fort Myers, Florida, USA, and nursed in an indoor recirculating system. Postlarvae were fed a commercial feed (Zeigler Bros. Inc. Gardners, PA, USA; protein ≥50%, fat ≥15%, fibre ≤1%) using an automatic feeder for ~1 week and then switched to crumbled commercial shrimp feed (Zeigler Bros. Inc., Gardners, PA, USA; protein ≥40%, fat ≥9%, fibre ≤3%) for ~1 week. Growth trial was conducted with three replicate tanks for each diet. Fifteen Pacific white shrimp were stocked per tank with mean initial weight of 0.17 ± 0.01 g. Test diets were offered four times daily for 8 weeks.

Daily feed ration was calculated based on expected growth of shrimp assuming a feed conversion ratio of 1.8 and a doubling in size (approximately every 7 days) until the estimated shrimp weight was in excess of 1 g. Thereafter, a growth rate of 1 g/week was assumed. Daily allowances of feed were adjusted based on observed mortality, weekly counts of the shrimp and observed feed consumption. At the conclusion, shrimp were group weighed and mean final biomass, final weight, survival, weight gain, feed conversion ratio were determined (Table 4).

2.4 | Haemolymph analysis

Samples of haemolymph were obtained from shrimp collected at the end of the trial. Haemolymph was withdrawn from shrimp via the pericardial cavity using a 25-gauge needle and 1-cc syringe inserted beneath the carapace at the cephalothorax-abdominal junction (Roy et al., 2009). The haemolymph was collected into a syringe preloaded with known quantity of anticoagulant (roughly in 1:1 ratio) to prevent clotting, and the dilution factor was calculated for each sample considering the ratio of each solution in the final sample (the weight of anticoagulant and haemolymph were determined gravimetrically). One composite sample of haemolymph per tank was prepared by collecting haemolymph from 3–4 shrimp in each tank. The anticoagulant solution (Liu et al., 2004) was prepared to contain 30 mM sodium citrate tribasic dihydrate (Sigma S4641); 0.34 M sodium chloride; 10 mM EDTA—ethylene diamine tetra acetic acid (Sigma, E9884); in de-ionized (DI) water. The haemolymph samples were immediately centrifuged (Fisher Scientific: Marathon 16 km, USA) at 1000 g for 15 min and the haematological parameters (alkaline phosphate, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin, urea nitrogen and cholesterol) were determined using VetScan VS2 analyzer (Abaxis, Inc. Union City, CA, USA). Furthermore, the total haemocyte count (THC) was determined using haemocytometer under a light microscope at 40x magnification (Mahasri et al., 2018) (Table 5).

Ingredient (g/100 g as is)	FCPC0	FCPC4	FCPC8	FCPC11	FCPC13	FCPC15
Menhaden fishmeal ^a	16.0	12.0	8.0	4.0	2.0	0.0
FCPC ^b	0.0	3.8	7.7	11.5	13.4	15.3
Soybean meal ^c	46.3	46.3	46.3	46.3	46.3	46.3
Corn protein concentrate ^d	1.0	1.0	1.0	1.0	1.0	1.0
Menhaden fish oil ^a	5.0	5.3	5.5	5.8	5.9	6.0
Lecithin ^e	1.0	1.0	1.0	1.0	1.0	1.0
Cholesterol ^f	0.1	0.1	0.1	0.1	0.1	0.1
Corn starch ^f	2.5	1.9	1.3	0.7	0.4	0.2
Whole wheat ^f	24.5	24.5	24.5	24.5	24.5	24.5
Mineral premix ^g	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix ^h	1.8	1.8	1.8	1.8	1.8	1.8
Choline chloride ⁱ	0.2	0.2	0.2	0.2	0.2	0.2
Stay C (35% active) ^j	0.1	0.1	0.1	0.1	0.1	0.1
CaP-dibasic ^k	1.0	1.5	2.0	2.5	2.8	3.0
Proximate composition ^l (g/100 g as is)						
Crude protein	36.7	36.8	36.2	37.1	36.7	37.0
Moisture	6.4	6.3	7.7	5.7	6.1	5.1
Crude fat	7.5	7.5	7.6	8.1	8.2	8.4
Crude fibre	3.8	3.9	3.7	4.3	3.9	3.9
Ash	7.6	7.4	7.0	7.0	6.8	6.8

^aOmega Protein Inc., Houston, TX, USA.

^bFCPC: Fermented corn protein concentrate sourced from Cargill, Minneapolis, MN, USA (MotivTM).

^cDe-hulled Solvent Extracted Soybean Meal, Bunge Limited, Decatur, AL, USA.

^dEmpyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

^eThe Solae Company, St. Louis, MO, USA.

^fMP Biomedicals Inc., Solon, OH, USA.

^gTrace mineral premix (g/100 g premix): cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.550; ferrous sulphate, 2.000; magnesium sulphate anhydrous, 13.862; manganese sulphate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193; alpha-cellulose, 69.664.

^hVitamin premix (g/kg premix): thiamin HCl, 4.95; riboflavin, 3.83; pyridoxine HCl, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 856.81.

ⁱVWR Amresco, Suwanee, GA, USA.

^jStay-C® (L-ascorbyl-2-polyposphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

^kVWR Amresco, Suwanee, GA, USA.

^lAnalysis conducted by University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) (Results are expressed on g/100g of feed as is, unless otherwise indicated).

TABLE 2 Formulation and chemical composition of test diets used in the growth trial (% as is)

2.5 | Astaxanthin analysis

Corn protein concentrate (CPC) and fermented corn protein concentrate (FCPC), three selected diets formulated with low (0%), medium (7.7%) and high (15.3%), inclusion levels of FCPC (FCPC0, FCPC8 and FCPC15) and a composite sample of six whole-body shrimp from each replicate tank fed FCPC0, FCPC8 and FCPC15 were analysed for astaxanthin by high-performance liquid

chromatography (HPLC) at Eurofins SF Analytical Laboratories, Wilson, NC, USA (Table 6).

2.6 | Statistical analysis

All the data were analysed using SAS (V9.4, SAS Institute, Cary, NC, USA). Growth performances, immunological parameters of

TABLE 3 Amino acid profile (g/100 g as is) of test diets composed of different levels of fermented corn protein concentrate (FCPC)

Amino Acid ^a	FCPC0	FCPC4	FCPC8	FCPC11	FCPC13	FCPC15
Alanine	1.77	1.85	1.88	1.94	2.00	2.03
Arginine	2.29	2.27	2.15	2.15	2.07	2.10
Aspartic acid	3.48	3.49	3.34	3.26	3.23	3.27
Cysteine	0.49	0.53	0.55	0.59	0.57	0.63
Glutamic acid	6.34	6.62	6.68	6.94	7.20	7.26
Glycine	1.87	1.77	1.60	1.48	1.45	1.37
Histidine	0.90	0.92	0.89	0.89	0.89	0.90
Hydroxylysine	0.07	0.08	0.07	0.07	0.05	0.08
Hydroxyproline	0.27	0.21	0.16	0.14	0.10	0.07
Isoleucine	1.65	1.68	1.64	1.67	1.64	1.65
Lanthionine	0.05	0.05	0.04	0.06	0.03	0.06
Leucine	2.72	2.97	3.16	3.45	3.55	3.71
Lysine	2.21	2.18	2.03	1.99	1.94	1.93
Methionine	0.65	0.66	0.64	0.63	0.63	0.64
Phenylalanine	1.71	1.80	1.82	1.92	1.95	1.98
Proline	1.99	2.10	2.24	2.38	2.35	2.51
Serine	1.24	1.29	1.24	1.35	1.72	1.48
Taurine	0.23	0.20	0.17	0.15	0.14	0.12
Threonine	1.27	1.29	1.24	1.24	1.29	1.28
Tryptophan	0.46	0.40	0.42	0.41	0.40	0.39
Tyrosine	1.18	1.22	1.26	1.36	1.39	1.42
Valine	1.81	1.84	1.80	1.83	1.77	1.81
Sum of amino acids	34.7	35.5	35.1	35.9	36.5	36.7

^aAnalysis was conducted by University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) (Results are expressed on g/100 g of feed as is, unless otherwise indicated).

TABLE 4 Response of juvenile shrimp (0.17 ± 0.01 g) fed with diets contained different levels of fermented corn protein concentrate (FCPC) over a 8-week experimental period. Values represented the mean of three replicates

Diet	FCPC0	FCPC4	FCPC8	FCPC11	FCPC13	FCPC15	PSD	ANOVA	Linear Regression	
								p-value	r-square	p-value
Biomass (g)	64.4	62.2	56.0	63.5	53.0	53.2	3.49	0.219	0.229	0.041
Final weight (g)	4.37	4.67	4.00	4.53	3.83	4.07	0.20	0.118	0.141	0.12
Weight gain (g)	4.20	4.47	3.80	4.37	3.70	3.93	0.21	0.176	0.133	0.169
Weight gain (%)	2497	2597	2244	2531	2118	2293	123	0.153	0.168	0.091
FCR	2.57	2.40	2.80	2.43	2.93	2.70	0.12	0.121	0.119	0.186
Survival	97.7	89.0	93.0	93.3	91.3	87.0	2.07	0.157	0.190	0.077

Weight gain = (final weight-initial weight)/initial weight $\times 100\%$.

Feed conversion ratio (FCR) = feed offered/ (final weight-initial weight).

PSD = pooled standard deviation.

haemolymph, free and total astaxanthin level of whole-body shrimp were analysed using one-way ANOVA to determine significant differences ($p < 0.05$) among treatments followed by the Tukey's multiple comparison test to evaluate significant differences between treatment means according to Steel and

Torrie (1980). Additionally, linear regression was performed to identify the relationships among percentage addition of FCPC to the diet and the growth variables, immunological parameters of shrimp haemolymph and the astaxanthin level of whole-body shrimp.

TABLE 5 Haematological parameters of shrimp fed diets composed of different levels of fermented corn protein concentrate (FCPC) during the trial

Diet	FCPC0	FCPC4	FCPC8	FCPC11	FCPC13	FCPC15	PSD	ANOVA	Linear regression	
								p-value	r-square	p-value
Total haemocyte count (THC)	23.0	38.0	33.9	48.5	46.7	52.8	14.21	0.559	0.236	0.048
Alkaline phosphate (ALP)	268.5	309.1	245.6	223.5	261.5	268.8	30.18	0.589	0.036	0.449
Alanine aminotransferase (ALT)	93.0	120.5	109.6	116.3	135.8	106.4	9.37	0.139	0.116	0.166
Gamma-glutamyl transferase (GGT)	4.2	5.0	4.9	5.2	4.8	4.9	0.71	0.959	0.023	0.545
Total bilirubin (TBIL)	0.4	0.4	0.4	0.4	0.4	0.4	0.02	0.348	0.012	0.962
Urea nitrogen (BUN)	3.1	2.5	3.1	2.6	1.8	1.8	0.44	0.363	0.193	0.068
Cholesterol (CHOL)	48.6	59.5	46.9	49.8	44.6	40.8	4.90	0.229	0.163	0.096

Values represented the mean of three replicates.

TABLE 6 Astaxanthin level¹ (µg/g) of corn protein concentrate (CPC), fermented corn protein concentrate (FCPC), test diets (with 0, 8 and 15% inclusion of FCPC) and shrimp (fed FCPC0, FCPC8 and FCPC15)

		Astaxanthin from Diesters ²	Astaxanthin from Monoesters ³	Free Astaxanthin ⁴	Total Astaxanthin ⁵
Ingredients	CPC	ND	ND	ND	ND
	FCPC	ND	ND	ND	ND
diets	FCPC0	ND	ND	ND	ND
	FCPC8	ND	ND	ND	ND
	FCPC15	ND	ND	ND	ND
Shrimp whole body	FCPC0	<2.0	<1.0	0.75 ± 0.01 ^a	1.84 ± 0.04 ^a
	FCPC8	2.15 ± 0.14	<1.0	1.09 ± 0.17 ^b	4.20 ± 0.27 ^b
	FCPC15	3.39 ± 0.12	1.75 ± 0.15	1.40 ± 0.07 ^c	6.54 ± 0.18 ^c
p-value [*]		—	—	0.001	<0.001

Abbreviation: ND = not detected.

Limit of quantitation (µg/g): 2 = 2.0, 3 = 1.0, 4, 5 = 0.5.

¹Analysis conducted by Eurofins SF Analytical Laboratories (Wilson, NC, USA).

^{*}One-way ANOVA was applied only on free and total astaxanthin levels of whole-body shrimp. Values with different superscripts within the same column are significantly different based on Tukey pairwise comparisons.

3 | RESULTS

Growth performances of juvenile *L. vannamei* treated with diets containing different levels of FCPC replacing fish meal are presented in Table 4. At the end of eight-week culture period, no significant differences were detected in biomass, mean final weight, weight gain, weight gain percentage, FCR and survival ($p > 0.05$) of shrimp, which ranged from 53.0–64.4 g, 3.83–4.67 g, 3.70–4.47 g, 2118–2597%, 2.4–2.9 and 87.0–97.7%, respectively (Table 4).

In line with the statistical outcomes in growth performance of shrimp (based on one-way ANOVA), no significant differences were noted in THC or rest of the haematological parameters of shrimp tested during the study (Table 5). However, as per the results of linear regression, a significant positive association was noted between the THC of shrimp and the percentage inclusion level of FCPC (0 to 15%) in diets tested during the study ($p = 0.048$; $r^2 = 0.24$).

No detectable level of astaxanthin was found in CPC, FCPC and the three selected test diets (FCPC0, FCPC8 and FCPC15) used during the study (Table 6). However, a significant ($p < 0.05$) increase in astaxanthin concentration (both free and total astaxanthin) was noted in shrimp whole-body corresponding to the increase in inclusion level of FCPC in the diet (Figure 1).

4 | DISCUSSION

The developments in ingredient manufacturing industry are proceeding at an increasing pace parallel to the accelerated growth in aquaculture feed production. As a result, numerous alternative plant-based protein sources have been tested and are available in the marketplace with diversified benefits such as efficacy of partial or complete replacement of fishmeal, competitive price, higher availability, probiotic or prebiotic effects, and sustainability. CGM and

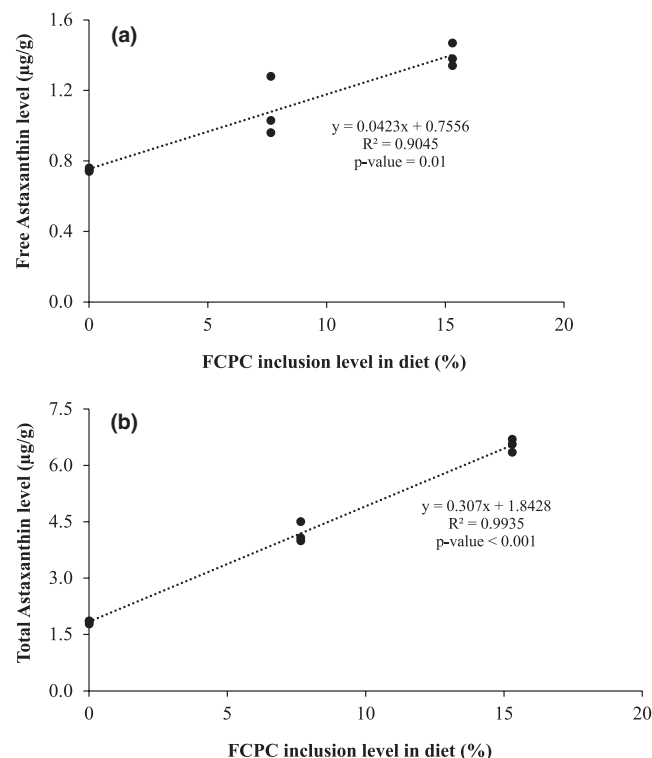


FIGURE 1 Relationship between fermented corn protein concentrate (FCPC) inclusion level in diet and astaxanthin level (a: free astaxanthin, b: total astaxanthin) in whole-body shrimp

CPC are popular high protein co-products used as feed ingredients in aquaculture feed formulations yield from corn milling industry, which are under continuous improvements due to the development in processing technologies, research and innovations (Table 1). The product (FCPC) tested during the study is one of the latest introductions to the sequence of corn-based feed ingredients.

During the current study, we demonstrated the potential of 100% replacement of FM using FCPC, without compromising the growth, FCR and survival of shrimp in clear water conditions. The inclusion level of FCPC in the diet devoid of FM was 15%, which had no significant negative effects on the performances of shrimp. This is an improvement in the level of inclusion compared with CGM and CPC, which impacted negatively on growth and feed consumption, at inclusion levels of 8.6 and 12%, respectively in the diet fed to *L. vannamei* in clear water systems (Zhou et al., 2014; Molina-Poveda et al. 2015).

One of the limitations of CGM and CPC responsible for the depressed growth of shrimp in FM replacement diets reported in the literature may have been due to a deficiency in essential amino acids, in particular lysine. The dietary lysine requirement for *L. vannamei* has been reported at 5.19% protein (Fox et al., 1995), although Xie et al., (2012) reported slightly lower need, around 4.9% protein. The lysine level of the diet devoid of FM (and 15% corn product) used during the current study was 5.2% of the protein, which was above the minimum requirement mentioned in the literature. Lysine content in FCPC is around 290% higher than that of CPC when compared on protein basis. In addition to lysine, glutamic acid, valine,

arginine, aspartic acid and glycine were reported high in FCPC compared with CPC and other corn-based feed ingredients, assumed to be due to the process of fermentation of the product (Table 1). Overall, the test diets formulated using fermented corn protein concentrate (FCPC), with no deficiencies in amino acids (Table 3), could be the reason for similar growth performances of shrimp on all diets observed during the study.

In addition to the elevation of ingredient digestibility, feed utilization and growth of shrimp, fermented products are encased with probiotic and antimicrobial characteristics to enhance the immune responses and disease resistance against pathogens (Phongpaichit et al., 2006). Elshopakey et al., (2018) reported an elevation in the innate immune response in kuruma shrimp, *Marsupenaeus japonicas* in terms of THC, phagocytosis, bactericidal activity and increased resistance against *Vibrio parahaemolyticus*, as a response to the dietary administration of yeast and *Lactobacillus* fermented vegetable product in the diet. Antimicrobial peptides derived from fermentation can also upregulate the immune- and stress-related gene expression (Ding et al., 2015). THC found to be strongly correlated with the capacity to encapsulate or phagocytose parasites (Bergin et al., 2003; Brayner et al., 2007; Eslin & Prévost, 1998; Ji et al., 2009) and therefore used as an indicator to assess the immune response status of shrimp. Based on linear regression outcomes, a marginally significant improvement was noted in total haemocyte count (THC) in shrimp response to the inclusion level of FCPC in the diet. This is assumed to be due to the probiotic properties of fermented corn, which proactively boost the disease resistance of shrimp.

In addition to THC, most of the blood parameters tested during the current study were indicative of liver and kidney disorders, which could be resulted due to both abiotic and biotic factors such as poor water quality, dietary issues such as antinutrients and nutrient imbalances, disease agents and many other factors (Agrahari et al., 2007). However, no significant differences were noted in the immunological parameters in shrimp haemolymph tested during the study, indicating the efficacy of higher inclusion levels of FCPC in the diet, which should be studied for further.

Astaxanthin is largely responsible for the red coloration of shrimp flesh after cooking which is an important criterion to increase the value of cultured shrimp by improving its visual appeal (Ju et al., 2011; Latscha, 1989). In addition to flesh pigmentation, astaxanthin has been reported to provide many positive biological effects for shrimp, such as enhancing survival, maturation, immune response, and reducing stress associated with high ammonia, low oxygen, temperature and salinity (Chien et al., 1999, 2003; Darachai et al., 1998; Merchie et al., 1998; Pan et al., 2001). An extensive array of experiments from around the world have established health benefits of natural astaxanthin on human as well, which include eye and brain health, UV protection and skin health, antioxidant and anti-inflammatory activity, immune system modulation and cardiovascular health (Capelli et al., 2013; Chien & Jeng, 1992; Guerin et al., 2003). Although shrimp are unable to biochemically synthesize astaxanthin within the body, it has been reported that some of the carotenoids such as lutein, zeaxanthin and β -carotene can be bio-converted by shrimp to astaxanthin (Nur-E-Borhan, 1993;

Meyers, 1994). Based on the product specification sheet from Cargill, Minneapolis, MN, USA, FCPC was reported to contain cis-lutein (56–81 ppm), trans-lutein (133–176 ppm), cis-zeaxanthin (44–69 ppm), trans-zeaxanthin (92–133 ppm), alpha-cryptoxanthin (2–4 ppm), beta-cryptoxanthin (9–12 ppm), alpha-carotene (13–19 ppm) and beta-carotene (23–27 ppm), but no astaxanthin. This was confirmed during the current study which showed no detectable level of astaxanthin in both corn-based ingredients (CPC and FCPC) used during the feed formulation and in the feed (FCPC0, FCPC8 and FCPC15). However, a significant ($p < 0.05$) increase in astaxanthin concentration (both free and total astaxanthin) was noted in shrimp whole-body corresponding to the increase in inclusion level of FCPC in the diet, could be due to the bioconversion of carotenoids that existed in FCPC, which should be studied for further.

5 | CONCLUSION

The present study suggest that the fermented corn protein concentrate (FCPC) could be used to replace 100% fishmeal, at an inclusion level high as 15%, without compromising the growth, feed conversion and survival of shrimp. A marginally significant improvement was noted in total haemocyte count (THC) of shrimp in respond to the inclusion level of FCPC in the diet, assumed to be due to the probiotic properties of FCPC. The significant increase in free and total astaxanthin concentration in shrimp whole-body corresponding to the increase in inclusion level of FCPC in the diet perhaps due to the bioconversion of carotenoids that existed in FCPC.

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DATA AVAILABILITY STATEMENT

The data associated with this manuscript entitled '**Fermented corn protein concentrate to replace fishmeal in practical diets for Pacific white shrimp *Litopenaeus vannamei***' are available at Dr. Allen Davis Laboratory, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, AL, USA, and could be accessed based on the permission of Dr Allen Davis (<https://sfaas.auburn.edu/davis/>).

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