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ORIGINAL ARTICLE

Evaluation of dietary corn fermented protein on growth performance and haemato-immunological parameters of the Pacific white shrimp *Litopenaeus vannamei*

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Abstract

The use of corn fermented protein (CFP) as a sustainable and functional ingredient in a shrimp diet formulation was evaluated. Four isonitrogenous and iso-lipidic practical diets were formulated to contain 0%, 6%, 12% and 18% of CFP obtained from mechanical separation of protein post distillation within dry-grind ethanol plants utilizing separation technology from Fluid Quip Technology. The 18% CFP diet was formulated to partially replace the use of fish meal (FM) and soybean meal (SBM) as well as completely replace the use of corn gluten meal (CGM). A total of 300 shrimp with average initial body weight of 2.02 ± 0.03 g were randomly stocked into four groups, with five replicates per group per treatment and 15 shrimp per aquaria tank. The results showed that in general, CFP could enhance the growth performance of shrimp. The use of 6% and 12% inclusion level of CFP resulted in a significant increase in final biomass, final mean weight (FMW), thermal growth coefficient (TGC) and superior feed conversion ratio (FCR) compared with the control diet ($p < 0.05$). Reducing the amount of FM from 10 to 7.5% and supplemented with CFP still have better FMW, weight gain (WG), FCR and TGC compared with the control treatment. No significant differences of total haemocyte count ($p = 0.2619$) and lysozyme activity ($p = 0.1185$) were observed. In addition, the protein content and protein retention efficiency showed no significant differences ($p > 0.05$) among the treatments. These results suggest that 18% corn fermented protein can be utilized as an alternative protein source without compromising the growth and health condition of shrimp *L. vannamei*.

KEYWORDS
corn fermented protein, growth, *Litopenaeus vannamei*, lysozyme activity, total haemocyte counts

1 | INTRODUCTION

The promotion of sustainable aquaculture system in the coming years will depend on the reduction in fish meal (FM) and an increased inclusion level of sustainable protein sources in the diet formulation (Novriadi, 2017; Novriadi & Davis, 2018; Novriadi et al., 2019). However, the presence of anti-nutritional factors (ANFs), such as lectins, phytic acid, saponins, phytosterols and allergens (NRC, 2011) within soybean meal (SBM) and toxic compounds, such as gossypol within cottonseed meal (CSM), may limit the wider use of several

plant-protein sources in aquafeed formulation as well as cause unfavourable physiological effects, such as depressing growth performance and induce histomorphological change in some species of fish (Barros et al., 2002; Nordrum et al., 2000; Novriadi et al., .2019; Rinchard et al., 2003). Consequently, there will most likely be a need to carry out further processing techniques to the conventional plant-protein sources to produce ingredients of higher nutritional value and a more digestible nutrient specification.

Dried distiller's grain with solubles (DDGS) as the predominant maize co-product produced by dry-grind fuel ethanol plants typically contain approximately 27% crude protein (CP), 42% neutral detergent fibre and 0.6% phosphorus (Stein & Shurson, 2009). They might have the potential to replace the use of FM and other plant-protein sources within diet formulations. In addition, the use of DDGS can be a promising, cheaper alternative to produce an economical diet compared with SBM on 'as per unit' of protein basis (Davis & Sookying, 2009). However, due to the inconsistency in terms of quality and results (Parsons et al., 2006), data have shown that traditional DDGS can only be used at moderate levels in rainbow trout diets (Cheng & Hardy, 2004) and not more than 10% in shrimp practical diets (Rhodes et al., 2015). Therefore, further processing to increase the protein level and reduce the indigestible fibre content results in a more attractive feed ingredient for aquafeed (Stone et al. 2005). The initial attempt was via front-end fractionation technology to separate the fermentable portion of the corn kernel from the non-fermentable portion prior to fermentation resulting in a higher protein, reduced fat and fibre when compared with traditional DDGs, this has been referred to as High Protein DDGs (Singh et al., 2005). The latest process advancement of the ethanol industry is post-fermentation mechanical separation of the whole stillage stream to concentrate the protein and fibre into separate DDG products. Fractionating the product post distillation allows the fermentation process to assist with separation, weaken the cellular wall structure of the fibrous fractions and concentration of inactive *Saccharomyces cerevisiae* yeast utilized for the production of alcohol. The industry is calling the protein fraction corn fermented protein (CFP). The commercial CFP (NexPro^{TM}, Flint Hills Resources Biofuels and Ingredients, KS, USA) which was evaluated in this study has a superior crude protein (~50% vs. ~28%) compared with traditional DDGS, lower crude fibre levels and improved nutritional composition to traditional DDGS. As a result, commercial CFP will likely compete with soy protein concentrate, corn protein concentrate, corn gluten meal and brewer's yeast as an ingredient in fish feed formulations. The use of CFP has been evaluated in shrimp *Litopenaeus vannamei*. From a series of trials performed by Qiu et al. (2017), CFP was considered a favourable plant-based ingredient and can be included up to 30% as a replacement of SBM without compromising the growth and feed utilization performance of shrimp. In addition, Qiu et al. (2017) suggested that in order to replace the combination of SBM and FM in the practical diet, up to 18% inclusion levels of CFP can be utilized without affecting the growth performance of shrimp cultured in controlled environment. Moreover, Guo et al. (2019) demonstrated the use of CFP containing an elevated level of yeast, can be used to replace corn protein concentrate or up to 15% inclusion of FM in shrimp diets. Currently, there is limited information on the use of CFP in low FM diets and to replace the combination of several plant-ingredients to enhance the growth and health status of the shrimp. Therefore, the purpose of this study is to evaluate the effect of various inclusion levels of CFP in low FM diets, partial replacement of SBM, and completely replace the use of corn gluten meal (CGM) on the growth and haemato-immunological parameters of *Litopenaeus vannamei* shrimp.

2 | MATERIALS AND METHODS

2.1 | Ethics Statement

All procedures were approved by the Committee on Animal Health Ministry of Marine Affairs and Fisheries, Republic of Indonesia and US National Research Council's "Guide for the Care and Use of Laboratory Animals".

2.2 | Experimental diets

Four iso-nitrogenous and iso-lipidic (37% protein and 8% lipid) experimental diets were formulated (Table 1). Basal diet was designed by utilizing 10% FM, 47.2% SBM (FKS Multi Agro), 17% wheat products (WP, FKS Multi Agro) and 8% CGM (FKS Multi Agro). Three experimental diets were formulated to utilize CFP (NexPro^{TM}, Flint Hills Resources) to be added into the basal diet at 6%, 12% and 18%. The fourth diet was designed to partially replace the use of FM and completely replace the use of CGM with 18% inclusion level of CFP. Proximate and amino acid composition of the primary protein sources used in this study is presented in Table 2. All experimental diets were produced at the Main Center of Mariculture Developme nt of Lampung (Lampung, Indonesia) following standard manufacturing procedures for making shrimp feed. Briefly, pre-ground dry ingredients and oil were weighed and mixed in a mixer. Hot water was then blended into the mixture to attain a consistency appropriate for pelleting. All experimental diets then subjected to steam-pelleting process with a 2- and 4-mm die, dried in a forced air oven (50°C) to a moisture level less than 10%. Dry pellets were crumbled, packed in sealed bags and subsequently stored in a freezer (4°C) until further use.

2.3 | Growth trials

Pacific white shrimp post larvae (PL) were obtained from a private commercial shrimp hatchery PT. Maju Tambak Sumur (Kalianda, Lampung, Indonesia) and nursed in a semi-indoor recirculating system. Water quality was maintained by recirculation through vertical sand filter (Dab Pumps S.p.A.). Dissolved oxygen was maintained near saturation using air stones in each culture tank

TABLE 1 Composition (% *as is*) of diets containing corn fermented protein (CFP) into the basal diet and fed to *L. vannamei* for 53 days

	Diet code			
Ingredients (% *as is*)	**Control**	**6% CFP**	**12% CFP**	**18% CFP**
Menhaden Fishmeal[1]	10.00	10.00	10.00	7.50
Soybean meal[2]	47.20	44.80	45.20	42.20
Corn Gluten Meal[2]	8.00	5.00	0.00	0.00
Corn Fermented Protein[3]	0.00	6.00	12.00	18.00
Menhaden fish oil[4]	5.22	5.14	5.05	5.10
Corn Starch[2]	6.38	5.86	4.55	4.00
Wheat products[2]	17.00	17.00	17.00	17.00
Mineral premix[5]	0.50	0..50	0.50	0.50
Vitamin premix[6]	1.80	1.80	1.80	1.80
Choline chloride[2]	0.20	0.20	0.20	0.20
Stay-C 35%[7]	0.10	0.10	0.10	0.10
Soy-lecitihin[2]	1.00	1.00	1.00	1.00
Cholesterol[2]	0.10	0.10	0.10	0.10
KP dibasic[2]	2.50	2.50	2.50	2.50
Proximate analysis (% *as is*)[8]				
Crude protein	37.13	38.58	38.49	38.56
Lysine	2.01	1.97	1.98	1.98
Methionine	0.82	0.80	0.82	0.78
Moisture	7.68	7.82	7.51	7.59
Crude Fat	8.13	8.38	8.72	8.95
Crude Fibre	3.56	3.49	3.63	3.76
Ash	6.15	6.90	5.64	6.30

[1] High protein fish meal (Peru) supplied by Agri Permata Asia, Jakarta, Indonesia.

[2] De-hulled solvent extract soybean meal, FKS Multi Agro, Jakarta, Indonesia.

[3] NexPro^{TM}, Flint Hills Resources, Wichita, KS, USA.

[4] Supplied by Bogor Ingredients, Indonesia.

[5] Trace 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.

[6] Vitamin 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.

[7] DSM Nutritional products.

[8] Analysis conducted by the SUA Integrated Fish Farm, Bogor Agricultural University, West Java, Indonesia.

and the sump tank using a common airline connected to a regenerative blower. PL were fed with a commercial feed (Evergreen Feed, Lampung, Indonesia) for 3 weeks until reached the suitable size. Shrimp (2.02 ± 0.03 g initial mean weight) were stocked into 70 × 35 × 40 cm (98 L) tank with 15 shrimp per aquarium per tank. Five replicate groups of shrimp were offered with experimental diets using nutrition research standard protocol for 53 days. Based on our historic results (Guo et al., 2019 and Qiu et al., 2017), feed inputs were pre-programmed assuming the normal growth of shrimp and employing a standard feed conversion ratio of 1.5. Daily allowances of feed were adjusted based on observed feed consumption and weekly counts of the shrimp.

2.4 | Water quality and growth response

For water quality analysis: pH, dissolved oxygen (DO), water temperature and salinity were measured four times daily using Aqua TROLL 500 Multiparameter Sonde instrument and connected to AquaEasy apps (Bosch, Singapore) for data monitoring and recording system. Total ammonia nitrogen (TAN) and nitrate were measured once a week using absorption spectrophotometry (DR890, HACH). At the end of feeding period, all shrimp were grouped and individually weighed to calculate the final biomass, final body weight (FBW), percentage of weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR) and thermal unit growth coefficient (TGC) as follows:

TABLE 2 Proximate and amino acid composition (% as is) of fish meal (FM), soybean meal (SBM), corn fermented protein (CFP) and corn gluten meal (CGM)

(g/100 g as is)	FM[1]	SBM[1]	CFP[2]	CGM[3]
Crude protein	64.98	46.21	49.20	58.25
Crude fat	8.02	0.82	3.11	4.74
Ash	12.68	6.22	4.87	1.46
Amino acids				
Serine	2.18	1.93	2.08	2.29
Glutamic acid	7.69	7.94	7.23	11.2
Phenylalanine	2.45	2.39	2.57	3.52
Isoleucine	2.58	2.14	2.19	2.23
Valine	3.08	2.21	2.87	2.42
Alanine	3.99	1.89	3.36	4.33
Arginine	3.82	3.49	2.30	1.66
Glycine	5.11	1.90	1.95	1.28
Lysine	4.92	2.91	2.01	0.93
Aspartic acid	5.38	5.04	4.05	2.97
Leucine	4.44	3.51	5.57	9.82
Tyrosine	1.92	1.68	2.01	2.86
Proline	3.00	2.15	3.33	4.93
Threonine	2.52	1.79	2.02	1.81
Histidine	1.66	1.22	1.33	1.32
Cysteine/cystine	0.51	0.68	0.97	1.01
Methionine	1.69	0.59	1.01	1.21
Tryptophan	0.65	0.62	0.43	0.27

Note: Nutritional composition data sourced from, [1]Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia, [2]Guo et al. (2019) and [3]Galkanda-Arachchige et al. (2021).

$$PWG = \frac{(average\ individual\ final\ weight - average\ individual\ initial\ weight)}{(average\ individual\ initial\ weight)} \times 100.$$

$$FCR = \frac{feed\ give\ (g)}{live\ weight\ gain\ (g)}.$$

$$SR = \frac{final\ number\ of\ fish}{initial\ number\ of\ fish} \times 100.$$

$$TGC = \frac{FBW^{\frac{1}{3}} - IBW^{\frac{1}{3}}}{\sum TD} \times 100,$$

where FBW is final body weight, IBW is initial body weight, T is water temperature (°C) and D is number of trial days.

2.5 | Total haemocyte count

At the end of growth trial, haemolymph was sampled from two intermolt shrimp per tank or 10 shrimp per treatment and total haemocyte count was determined. Haemolymph (100 µl) of individual shrimp was withdrawn from the pleopod base of the second abdominal segment with a sterile 1-ml syringe (25 G × 13 mm needle). Before haemolymph extraction, the syringe was loaded with a precooled (4°C) solution (10%-EDTA, Na2) used as an anticoagulant. The haemolymph with anti-coagulant solution was diluted in 150 µl of formaldehyde (4%) and then 20 µl was placed on a haemocytometer (Neubauer) to determine the total haemocyte count (THC) using an optical microscope (Olympus, DP72).

2.6 | Lysozyme activity analysis

Lysozyme activity was measured using a lysozyme detection kit (Sigma-Aldrich, Cat. no. LY0100) according to the manufacturer's instructions. The results of lysozyme activity were defined by the lysis of the *Micrococcus lysodeikticus* cells, gram-positive bacteria that is normally highly sensitive to lysozyme. The reactions were conducted at 25°C and absorbance at 450 nm was measured on the ultraviolet/visible spectrophotometer (Perkin Elmer, Lambda XLS). The absorbance of the samples was then compared with the absorbance of the blank.

$$\text{Lysozyme activity}\left(\frac{\text{Units}}{\text{mL}}\right) = \frac{(\Delta A450/\min Test - \Delta A450/\min Blank)\,(df)}{(0,001)\,(0.03)}$$

df = dilution factor.

0.001 = ΔA_{450} as per the unit definition

0.03 = Volume (in mL) of enzyme solution.

2.7 | Protein level and retention analysis

Upon termination of the trial, four shrimp from each tank (i.e. 20 per treatment) were randomly sampled and stored at −60°C for body composition analysis. Prior to the protein analysis, dried whole shrimp were rigorously blended and chopped in a mixer according to the standard methods established by Association of Official Analytical Chemists (AOAC, 1990). Protein content of whole shrimp body was analysed using Kjeldahl method and conducted at PT. Angler BioChem Lab (Surabaya, East Java, Indonesia). Protein retention rate (%) was calculated using the following formula:

$$\text{Protein retention efficiency}\,(\%) = \frac{(final\ weight^{*}final\ protein\ \%) - (initial\ weight^{*}ini.protein\ \%)}{total\ protein\ intake\ (dry\ matter)} \times 100.$$

2.8 | Statistical analysis

All data were analysed using a one-way analysis of variance to determine the significant difference ($p < 0.05$) among the treatment means followed by the Tukey's multiple comparison test to determine difference between treatments means in each trial. The pooled standard errors were used across all the growth parameters as the variance of each treatment is the same. Statistical analyses were conducted using SAS system (V9.4, SAS Institute).

3 | RESULTS

3.1 | Water quality

The overall mean and standard deviation of morning and afternoon pH, salinity (‰), water temperature (°C) and dissolved oxygen (mg/L) together with Total ammonia nitrogen (mg/L) and Nitrate (mg/L) are displayed in Table 3. All these parameters remained within the acceptable range for *L. vannamei*.

3.2 | Growth performance

Growth performance and survival of Pacific white shrimp *L. vannamei* fed with the experimental diets are displayed in Table 4. Final mean weight and TGC of shrimp fed with 6%, 12% and 18% CFP were significantly higher compared with the control diet ($p < 0.05$). Meanwhile, shrimp fed 6% and 12% CFP had the greatest biomass compared with the control diet ($p = 0.0174$). In addition, FCR of shrimp fed 6%, 12% and 18% CFP were significantly lower than the shrimp fed with the control diet ($p = 0.0111$), whereas no significant difference was detected in the survival of shrimp among all treatments during the growth trial.

3.3 | Haemato-immunological parameters

Total haemocyte counts and lysozyme activity of the shrimp fed with various inclusion levels of CFP are shown in Figures 1 and 2 respectively. Statistically, there is no difference in terms of THC among the treatments ($p = 0.2619$). With regard to the lysozyme activity, no significant differences were noted during the study.

3.4 | Protein level and protein retention rate

The protein level and protein retention efficiency as the effect of various inclusion level of CFP are shown in Figure 3. No significant differences in terms of protein level within the whole body of shrimp and protein retention rate (%) with regard to the substitution of FM, SBM and CGM with various inclusion level of CFP were observed ($p > 0.05$).

4 | DISCUSSION

A further processing technique of DDGS to produce CFP, increased crude protein, moderate level of yeast and low level of indigestible fibre, provides an attractive solution to enhance the nutritional quality and energy of plant-based ingredients and growth performance of shrimp *L. vannamei* (Guo et al., 2019). An added benefit of CFP compared with DDGS is that CFP contains a greater concentration of yeast which could help to enhance the disease resistance in shrimp due to enhanced functional characteristics. Yeast contains β-glucans which have the ability to stimulate non-specific components of the immune system (Burgents et al., 2004), thus opening the opportunity to produce a functional feed to overcome the disease concerns in shrimp via oral administration of these bio-active properties. This

TABLE 3 Overall water quality measurements during the grow-out phase of the experiment. Data were presented as mean ± standard deviation (range)

	Parameter					
Time	Temperature (°C)	D.O (mg L^{-1})	pH	Salinity (‰)	Ammonia (mg TAN L^{-1})	Nitrate (mg NO_2-N L^{-1})
AM	27.92 ± 067	5.71 ± 0.35	7.79 ± 0.07	25.19 ± 2.24	0.21 ± 0.18	33.25 ± 8.44
PM	29.34 ± 0.65	5.82 ± 0.62	7.73 ± 0.34	23.93 ± 7.62		

TABLE 4 Growth performance of Pacific white shrimp *Litopenaeus vannamei* (Mean initial weight 2.02 ± 0.03 g) fed experimental diets for 53 days

Diet code	Final Biomass (g)	Initial Mean Weight (g)	Final Mean Weight (g)	Survival (%)	WG[1] (%)	FCR[2]	TGC[3]
Control diet	121.18[a]	2.02	9.50[a]	85.33	368.15[a]	2.25[a]	0.0560[a]
6% CFP	143.87[b]	2.03	10.42[b]	92.00	411.63[ab]	1.98[b]	0.0603[b]
12% CFP	147.50[b]	2.01	10.41[b]	94.67	414.71[ab]	1.98[b]	0.0604[b]
18% CFP	141.36[ab]	2.03	10.40[b]	90.67	419.35[b]	1.99[b]	0.0607[b]
p-value	0.0174	0.5303	0.0138	0.4163	0.0307	0.0111	0.0191
PSE[4]	2.0517	0.0173	0.4009	1.7083	3.0092	0.2119	0.0287

Note: Values represent the mean of five replicates. Results in the same columns with different superscript letters are significantly different ($p < 0.05$) based on analysis of variance followed by Tukey's multiple comparison test.

[1]WG, Weight gain; [2]FCR, Feed conversion ratio; [3]TGC, Thermal growth coefficient; [4]PSE, Pooled standard error.

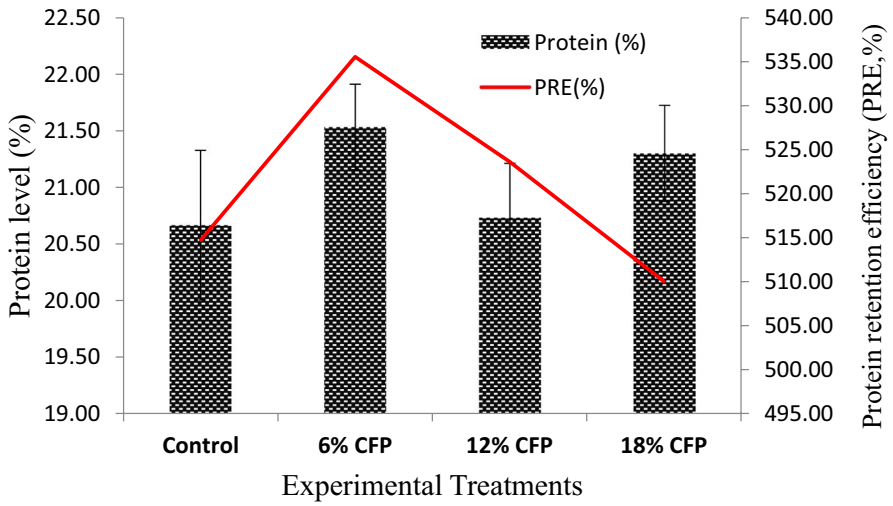

FIGURE 1 Protein level and protein retention efficiency (%) of whole body of Pacific white shrimp *Litopenaeus Vannamei* fed experimental diets for 53 days. Values represent the mean of five replicates ($n = 5$)

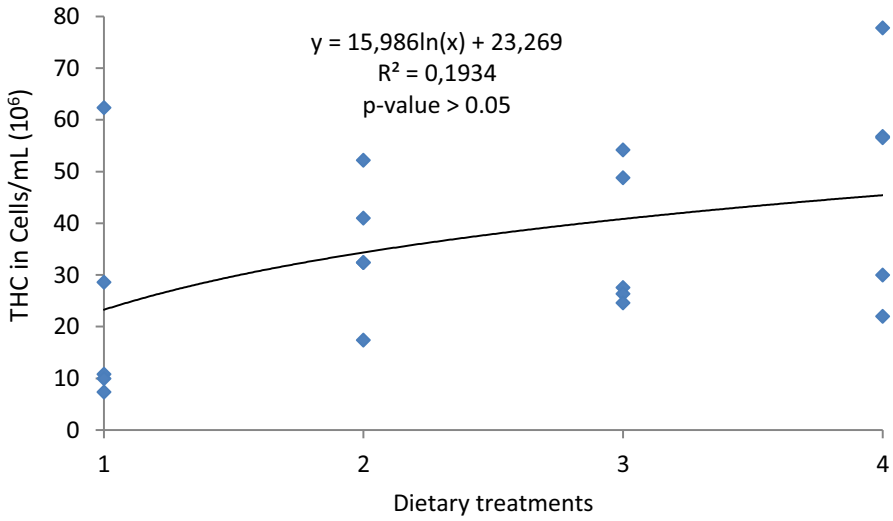

FIGURE 2 Relationship between corn fermented protein (CFP) inclusion level in the diet and total haemocyte count (THC) of Pacific white shrimp *Litopenaeus Vannamei* (10^6 cell/ml) at the end of the growth trial. Values represent the mean of five replicates. Dietary treatments 1 = control; 2 = 6% CFP, 3 = 12% CFP and 4 = 18% CFP

research describes the effect of using various inclusion level of CFP to enhance the growth and haemato-immunological parameter in shrimp *Litopenaeus vannamei*.

In the present study, partial replacement of SBM, CGM and FM as well as complete replacement of CGM with CFP significantly affects the final biomass, final mean weight, weight gain, thermal growth coefficient (TGC) and feed conversion ratio (FCR) of shrimp ($p < 0.05$). The inclusion of 6% and 12% of CFP to partially replace SBM and CGM and completely replace CGM were able to enhance the growth of shrimp compared with the control group. It is also important to note that the inclusion of 18% CFP providing complete replacement of CGM and partially replacing FM and SBM improved the final mean weight, TGC and FCR compared with the control group. This observation is in accordance with Guo et al. (2019) reporting that the use of 20% CFP can be effectively used to substitute the deletion of 50% corn protein concentrate (CPC) in the diet formulation. With regard to the replacement of FM, 15% CFP can be incorporated into shrimp diets to substitute 50% of FM without compromising the growth of Vannamei (Guo et al., 2019). Furthermore, Qiu et al. (2017) suggest that 18% inclusion level of CFP could be set as the upper limit that should be used in shrimp feed composition. Positive contributions of CFP to improve the growth of aquatic organisms could be attributed to the residual yeast, *Saccharomyces cerevisiae*, which is known to be an excellent source of essential amino acids and provide more optimal nutrition to the aquatic organisms (Burgents et al., 2004; Guo et al., 2019; Øverland et al., 2013).

During the growth trial, shrimp did not exhibit a negative response to palatability of the diet. This is indicated by the lower feed conversion ratio (FCR) for shrimp fed various concentrations of CFP compared with the control. Qiu et al. (2017) also found no adverse effect on the FCR of *L. vannamei* when they partially replace the use of FM and SBM. In addition, Guo et al. (2019) also demonstrated the insignificant difference in terms of FCR in shrimp fed with up to 20% of CFP to partially replace the use of corn protein concentrate (CPC) and FM as well as complete replacement of CPC with CFP. However, using CFP for more than 20% to partially replace FM and SBM, the FCR become less efficient compared with the basal diet (Qiu et al., 2017). Based on the facts above, further study by extending the growth trial period or conducting the growth trial within a commercial system is needed to validate the efficacy of CFP to improve the efficiency in feed utilization.

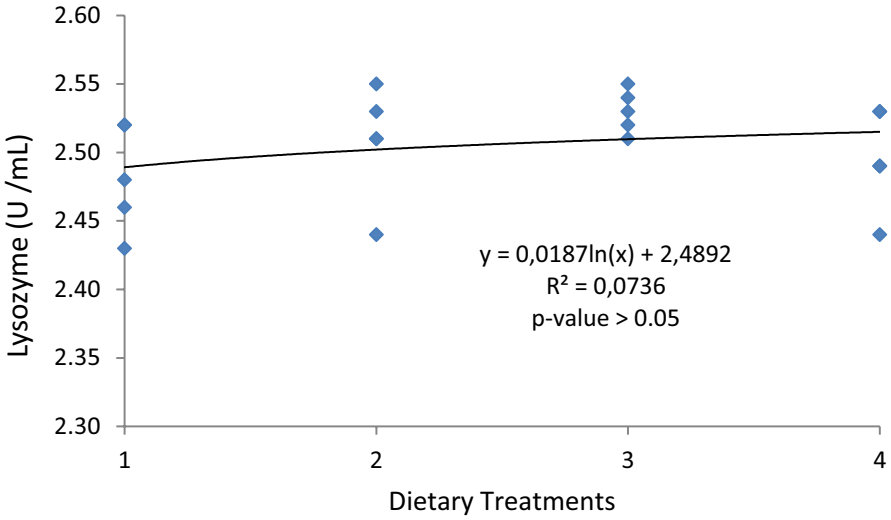

FIGURE 3 Relationship between corn fermented protein (CFP) inclusion level in the diet and lysozyme activity of Pacific white shrimp *Litopenaeus Vannamei* (U/ml) at the end of the growth trial. Values represent the mean of five replicates Dietary treatments 1 = control; 2 = 6% CFP, 3 = 12% CFP and 4 = 18% CFP

The results of this study indicate that the various inclusion levels of CFP do not affect the protein level and retention efficiency (%) in the whole body of shrimp. Similar responses were also observed when diets were designed to be iso-nitrogenous and iso-lipidic using various inclusion levels of CFP to partially replace the use of SBM and FM in the diet (Qiu et al., 2017). Dietary CFP could provide sufficient levels of protein compared with SBM and CGM and the inclusion of 12% could be used in shrimp feed diet formulation and lead to numerically better results compared with the control diet.

In shrimp culture, research on the use of functional ingredients to induce the activation of cellular and humoral immune defences is gaining attention and expanding (Kumar et al., 2016; Lee et al., 2020; Wu et al., 2016). Since the first immune process is the recognition of microorganisms mediated by plasma protein and haemocytes (Söderhäll & Cerenius, 1998), the quantitative measurement of total haemocyte count (THC) will explore the capability of functional ingredients to induce the activation of the non-specific immune system in shrimp. The evaluation of haemato-immunological parameter in this study revealed that the inclusion of CFP contain with 20–25% yeast, numerically, could enhance the THC and lysozyme activity compared with the control treatment. In crustacean, haemocytes, that has generally classified into three categories, hyaline cells, small granular cells and large granular cells, are an important component of the immune response and involved in non-self-recognition, phagocytosis, reactive oxygen intermediate production, wound repair and melanization with encapsulation of foreign materials (Martin et al., 1993; Muñoz et al., 2002). Meanwhile, Lysozyme activity acts as a non-specific immune system in shrimp that have been shown to possess lytic activity against a range of gram-positive and gram-negative bacterial species, including pathogenic *Vibrio* spp (De-La-Re-Vega et al., 2006; Hikima et al., 2003). Since, until recently, there is limiting study evaluating the effect of CFP to enhance the non-specific immune system and lysozyme activity in shrimp. This study may contribute to describe the efficacy of CFP on non-specific immune system of *L. vannamei* cultured under controlled environment.

5 | CONCLUSIONS

We have developed a practical diet for *L. vannamei* containing CFP as a sustainable functional ingredient to replace the use of FM, SBM and CGM. From the results of this study, it can be concluded that the use of CFP to a level up to 18% is acceptable and could promote the growth of Pacific white shrimp *L. vannamei.* The use of CFP had no adverse effect on THC and lysozyme activity. It would be interesting to validate the efficacy of CFP in various specific pathogen challenge studies and assess the cost-benefit of using CFP in complete feeds for shrimp cultured in commercial pond farming system.

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CONFLICT OF INTEREST

Ben Seiler, Derek Balk and Dr. Melissa Jolly-Breithaupt are employed by Flint Hill Resources. The rest of the authors state that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Romi Novriadi : Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Supervision; Writing original draft of manuscript. Aldy Eka Wahyudi: Formal analysis; Investigation; Methodology; Resources. Rifqi Gadhilah: Formal analysis; Investigation; Methodology; Resources. Ben Seiler: Data curation; Formal analysis; Investigation; Methodology; Resources; Supervision; Writing original draft of manuscript. Derek Balk: Data curation;

Formal analysis; Investigation; Methodology; Resources; Supervision; Writing original draft of manuscript. Melissa Jolly-Breithaupt: Data curation; Formal analysis; Investigation; Methodology; Resources; Supervision; Writing original draft of manuscript.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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