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Evaluation of distiller's dried grains with solubles in diets for Pacific white shrimp, *Litopenaeus vannamei*, reared under pond conditions

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Abstract

Most studies evaluating the use of distiller's dried grains with solubles (DDGS) to partially replace the use of soybean meal (SBM) in diets of shrimp *Litopenaeus vannamei* have been carried out under laboratory conditions, which has significant differences in terms of environmental and culture conditions with the out-door commercial pond. This study aims to extrapolate the laboratory trials into the out-door pond condition in order to evaluate the feasibility of incorporating DDGS at various levels (D0 (0%), D5 (5%), D10 (10%), and D 15 (15%)) to replace the use of SBM for 90-day culture period on the growth, body composition, total hemocyte count, lysozyme activity, and organoleptic characteristics of the shrimp. Seven hundred twenty shrimp (mean initial weight 1.06 ± 0.01 g) were randomly distributed into each of 40 net pens (3 × 2 × 1 per net pen) with 10 replicates per dietary treatment. The results showed an enhancement in biomass, mean weight, and thermal growth coefficient of shrimp fed with 5 and 10% inclusion levels of DDGS. Significant reduction of FCR was also observed with the use of 5 and 10% DDGS to partially replace SBM. There were no severe changes in the total hemocyte count (THC), lysozyme activity, color, aroma, and flavor of the shrimp. However, the texture of shrimp was significantly better with the use of 10 and 15% DDGS in the diet. In summary, regarding all the factors, DDGS is a promising alternative ingredient to replace the use of SBM with 5 and 10% inclusion levels and could modulate better growth, quality, and maintain the health condition of shrimp cultured in out-door pond conditions.

KEYWORDS
distiller's dried grains with solubles, growth, health, *Litopenaeus vannamei*, soybean meal

1 | INTRODUCTION

In an intensive Pacific white shrimp *Litopenaeus vannamei* production system, the development of economical diets that fulfills the specific nutrient requirement is needed to support the optimization of growth performance, health condition, and profitability of shrimp during the production period (Ayisi et al., 2017; NRC, 2011; Lim & Akiyama, 1995; Molina-Poveda, 2016; Sookying et al., 2013). Hence, the identification and evaluation of alternative ingredients became one of the important steps in the development of effective diets (Bai et al., 2022; Glencross, 2016; Yang et al., 2009). Traditionally, fish meal (FM) has been used in all aquaculture feeds as the primary protein source, including in shrimp, mainly because of the excellent source of micronutrients, vitamins, and mineral as well as the characteristics of high digestibility, palatability, attractability, lack of anti-nutritional factors (ANFs), and their effects on growth promotion (Council, 2011; Davis & Arnold, 2000; Samocha et al., 2004; Swick et al., 1995; Tantikitti, 2014). However, while alleviating concerns about sustainability of the industry and increasing prices of FM (Malcorps et al., 2019; Sarker et al., 2018), the amount of FM in feed formulation has begun to decrease significantly. Globally, shrimp feed manufacturers have reduced the inclusion of FM from a range of 19%–40% in the year of 2000 to 11%–23% in 2014 (Davis & Arnold, 2000; Malcorps et al., 2019). Even, Browdy et al. (2006) and Sookying and Davis (2011) have started to produce shrimp using alternative protein sources to completely replace FM in feed formulation to achieve an optimum productivity.

Among alternative protein sources, soybean meal (SBM) is commonly used to replace dietary FM as an economically and nutritious alternative ingredient (Lech & Reigh, 2012; Sales, 2009). This is because of the characteristics of SBM that has favorable amino acid profile and good digestibility properties, widely available, and can be easily shipped to reach the production center (Amaya et al., 2007; Davis & Arnold, 2000b; Gatlin et al., 2007). However, the presence of ANFs, such as phytic acid, saponins, phytosterols, lectins, and allergens (NRC, 2011), which are responsible for the decreased growth performance and feed efficiency of shrimp, may inhibit the wider use of SBM in diet formulation (Dersjant-Li, 2021; Jannathulla et al., 2017). Furthermore, the rising cost of SBM has driven the shrimp industry to search for other economical and sustainable alternatives for effective formulation and production purposes.

In shrimp industry, the use of DDGS has received significant interest as a feed ingredient, not only because of the nutritional profile but also the prices (Gyan et al., 2022; Novriadi, Suwendi, & Tan, 2022; Rhodes et al., 2015). The proper blending strategy with other protein sources makes DDGS a promising alternative to replace SBM (Novriadi et al. 2022). Recently, the production of DDGS increased significantly following the increasing number of dry-grind ethanol production facilities (Han & Liu, 2010). Each 100 kg of corn fermented in a dry-grind ethanol plant is able to produce approximately 36 liters of ethanol, 32 kg of DDGS, and 32 kg of carbon dioxide (Shurson & Noll, 2005; Welker et al., 2014). DDGS obtained from this process could contain with moderate level of protein (28%–33%), crude fiber (5%–11%), and low levels of starch. Furthermore, considering the lower cost of DDGS compared with SBM (on a per unit protein basis), the feed cost could to be reduced by 1.04%–3.05% as the inclusion of DDGS increases from 5 to 15% to partially replace the use of SBM (Novriadi, Suwendi, & Tan, 2022).

Previous results from a two-stage growth trial under a controlled environment showed that DDGS could be included in shrimp diet formulation to partially replace the use of soy-protein up to 15% without causing any adverse effect on the growth, feed conversion, nutrient utilization efficiency, and whole body composition (Novriadi, Suwendi, & Tan, 2022). However, limited information is available on the use of DDGS in *L. vannamei* reared in out-door ponds similar to commercial grow-out conditions. Therefore, the present study aims to evaluate the effect of incorporating varying inclusion levels of DDGS to partially replace the use of SBM on the growth performance, nutritional profile of the whole body, total hemocyte, lysozyme activity, and organoleptic characteristics of Pacific white shrimp *Litopenaeus vannamei* reared in out-door pond conditions.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

The formulation was designed to reduce the inclusion levels of soybean meal, while increasing the inclusion levels of DDGS in shrimp feed formulation. All test diets were formulated to be iso-nitrogenous and iso-lipidic (40% protein and 7% lipid). In this research, basal diet (D0) was formulated with 25% SBM, 8% FM, 20.3% poultry by-product meal (BPM), and 31.9% wheat flour (WF) as the primary ingredients. Three experimental diets were formulated to include various inclusion levels of corn DDGS (US Grains Council, USA) to partially replace SBM. The first experimental feed (Code: D5) was formulated to contain 5% corn DDGS to replace the use of SBM, and the second and third experimental diets were designed to contain 10 and 15% corn DDGS, respectively, to replace the use of SBM (designated as D10 and D15). All experimental diets were produced at the *Main Center* of *Mariculture* Development of *Lampung* using standard procedures for producing pelleted shrimp feed (Table 1). The proximate and amino acids (AA) profile of the diets were analyzed at Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia and summarized in Table 2.

2.2 | Growth trials

The growth trial was conducted in 40 net pens with size of 3 × 2 × 1 m per hapa net and installed in four commercial ponds with size of 25 × 10 × 1.6 m per pond at the Marine Science Techno Park, Diponegoro University (Semarang, Indonesia). A total of 28,800 juvenile Pacific white shrimp were obtained from PT. Riz Samudera (Jepara, Central Java, Indonesia) and acclimated to the culture system until they reached a suitable size. Seven hundred twenty shrimp (mean weight ± standard deviation, 1.06 ± 0.01 g) were then randomly distributed into each net pen (size of 3 × 2 × 1 m per net pen). Ten replicate groups of shrimp were administered different types of experimental diets using nutrition research standard protocol for 90 days and fed by hand four times daily, at 07:00, 11:00, 15:00, and 20:00 h. Feed inputs were pre-programmed based on our historical analysis assuming the normal growth of shrimp at the out-door pond condition and FCR of 1.5.

2.3 | Water quality and growth sampling

Water parameters, including dissolved oxygen (DO), pH, water temperature, and salinity, were measured four times daily using real-time sensors (Aqua TROLL 500 Multiparameter Sonde instrument) and connected to AquaEasy apps (Bosch, Singapore) for online data recording system. Parameters of total ammonia-nitrogen (TAN), nitrate, nitrite, and

TABLE 1 Composition (% *as is*) of diets consisting of fermented corn protein concentrate in commercial diet formulation to replace fish meal and poultry meal and fed to *Litopenaeus vannamei* for 90 days.

	Diet			
Ingredient (%)	**D0**	**D5**	**D10**	**D15**
Soybean meal[a]	25.00	22.50	20.00	17.50
Poultry by-product meal[b]	20.30	20.30	20.30	20.30
Fish meal[c]	8.00	8.00	8.00	8.00
Corn DDGS[d]	0.00	5.00	10.00	15.00
Tuna hydrolysate[e]	2.00	2.00	2.00	2.00
Squid liver powder[f]	6.00	6.00	6.00	6.00
Wheat flour[g]	31.90	29.30	26.80	24.20
Soy lecithin[h]	1.50	1.50	1.50	1.50
Fish oil[i]	1.00	1.00	1.00	1.00
Monocalcium phosphate[j]	1.80	1.80	1.80	1.80
L-lysine[k]	0.00	0.04	0.09	0.14
DL-methionine[l]	0.19	0.18	0.17	0.17
L-threonine[k]	0.08	0.08	0.08	0.09
Mineral premix[m]	1.20	1.20	1.20	1.20
Vitamin premix[m]	0.41	0.41	0.41	0.41
Magnesium sulphate[n]	0.35	0.35	0.35	0.35
Choline chloride[k]	0.20	0.20	0.20	0.20
Antimold[o]	0.12	0.12	0.12	0.12

[a]Dehulled Solvent Extracted Soybean Meal, Argentina.
[b]Griffin Industries, USA.
[c]Foodcorp, S.A., Chile.
[d]US Grains Council, USA.
[e]SPF Diana, Thailand.
[f]Hana Industrial Co. Ltd, South Korea.
[g]PT Agristar Grain, Indonesia.
[h]Shandong Maowei International Trade Co. Ltd., China.
[i]Pasquera La Portada S.A., Chile.
[j]Sinochem YunLong, Co. Ltd, China.
[k]PT Dian Cipta Perkasa, Indonesia.
[l]Evonik Nutrition & Care GmbH, Hanau, Germany.
[m]PT DSM Nutritional Products, Indonesia.
[n]PT Jannisika Sumber Jaya, Indonesia.
[o]PT Tienyen International, Indonesia.

phosphate were measured once in a week using absorption spectrophotometry (HACH, USA) and were conducted at the Main Center for Brackishwater Aquaculture Development (Jepara, Central Java, Indonesia). At the end of feeding period, all shrimp were grouped and randomly weighed to calculate the final biomass, final body weight (FBW), percentage of weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR), thermal unit growth coefficient (TGC), and average daily growth (ADG) as follows:

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

TABLE 2 Proximate, calories, and amino acid (AA) composition (% *as is*) of experimental diets utilized in the trial.

	Diet code			
	D0	D5	D10	D15
Proximate analyses (%, *as is*)[a]				
Protein content	38.96	38.64	38.61	38.60
Fat content	6.96	6.71	7.02	7.14
Moisture	11.24	10.96	8.82	8.48
Crude fiber	1.54	1.52	1.44	1.67
Calories (kcal/100 g)[a]				
Calories from Fat	53.64	51.39	64.98	65.00
Total calories	324.40	328.15	364.78	368.56
Amino acid profile (%, *as is*)[a]				
L-Serine	2.69	2.64	2.56	2.56
L-Glutamic acid	5.17	5.15	5.77	5.80
L-Phenylalanine	2.64	3.14	2.63	2.68
L-Isoleusine	1.57	1.47	1.53	1.49
L-Valine	1.92	1.77	1.84	1.83
L-Alanine	2.02	1.90	2.06	2.08
L-Arginine	3.04	3.40	2.96	2.98
Glycine	2.79	2.92	2.78	2.83
L-Lysine	1.93	1.52	1.78	1.68
L-Aspartic Acid	3.24	2.63	3.05	2.94
L-Leusine	3.01	2.89	3.04	3.04
L-Tirosine	1.60	1.87	1.51	1.58
L-Proline	2.73	2.60	2.76	2.76
L-Threonine	2.17	2.13	1.98	2.05
L-Histidine	1.15	1.23	1.11	1.14
L-Systine	0.68	0.68	0.71	0.62
L-Methionine	0.23	0.20	0.20	0.20
L-Tryptophan	0.34	0.30	0.33	0.32

[a]Analysis conducted by the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia. Website www.siglaboratory.com.

$$\mathrm{FCR} = \frac{\text{feed given (g)}}{\text{alive weigh gain in shrimp (g)}}$$

$$\mathrm{SR} = \frac{\text{final number of shrimp}}{\text{initial number of shrimp}} \times 100$$

$$\mathrm{TGC} = \frac{\text{Final body weight}^{1/3} - \text{Initial Body Weight}^{1/3}}{\sum \text{Temperature} \times \text{number of trial days}} \times 100$$

$$\mathrm{ADG} = \frac{\text{Total weight gained by the shrimp}}{\text{Total days of culture}}$$

2.4 | Body composition analysis

Two shrimp per hapa net or 20 shrimp for each dietary treatment were randomly sampled at the end of the growth for body composition analysis. Prior to analysis, dried whole shrimp were rigorously blended and chopped in a mixer according to the methods described by Association of Official Analytical Chemists (AOAC, 1990). All parameters were analyzed at the accredited laboratory of Saraswanti Indo Genetech Laboratory (Bogor, West Java, Indonesia).

2.5 | Total hemocyte count

Hemolymph was sampled from two intermolt shrimp per net or 20 shrimp per treatment, and total hemocyte count was determined. Hemolymph (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 hemolymph extraction, the syringe was loaded with a precooled (4°C) solution (10%-EDTA, Na2) used as an anticoagulant. The hemolymph with anticoagulant solution was then diluted in 150 μL of formaldehyde (4%) and then 20 μL of solution was placed on a hemocytometer (Neubauer) to determine the total hemocyte count (THC) using an optical microscope (Olympus, DP72).

2.6 | Lysozyme activity analysis

The measurement of lysozyme activity was conducted with 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. The absorbance was measured at 450 nm with ultraviolet/visible spectrophotometer (Perkin Elmer, Lambda XLS, USA).

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

df = dilution factor.

0.001 ΔA_{450}, as per the unit definition.

0.03 = Volume (in mL) of enzyme solution.

2.7 | Organoleptic analysis

Organoleptic evaluations were carried out at the Diponegoro University (Semarang, Indonesia). First, bags of shrimp were inspected to detect any damage and stored at −34°C in a freezer. 24 h before organoleptic evaluation, shrimp were thawed at 0–4°C in a refrigerator. Shrimp were inspected for general appearance and color criteria according to organoleptic assessment (Rachman et al., 2020). Shrimp were then weighed individually using a digital scale and individuals from the same treatment were cooked based on the protocol described by Brookmire et al. (2013). Shrimp were then cooled for approximately 1 min in a bowl of icy water and then manually peeled. Three shrimps were placed in the center of a dish and panelists received four packages of shrimp at a time. The evaluation for each panelist covered scoring the color, aroma, flavor, and texture on a nine-level hedonic scale as well as commenting freely their perceptions. All assessments took place in booths that respected global standards for the design of organoleptic analysis rooms. The light was of daylight type at an average intensity of 780 lux. Panelists were recruited from Diponegoro University (Semarang, Indonesia) and can be defined as an "internal trained panel" as they regularly consumed marine products, and already experienced in organoleptic tests.

2.8 | Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the growth performance proximate composition and AA profile of the whole body, THC, lysozyme, and organoleptic level to determine significant differences among treatments followed by Tukey's multiple comparison tests to determine the difference between treatment means among the treatments. Score data for organoleptic analysis were treated as categorical data, tested for normality and homoscedasticity and subsequently analyzed using linear regression model. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC, USA).

3 | RESULTS

3.1 | Water quality

Data for water quality, including temperature, dissolved oxygen (DO), salinity, pH, ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and phosphate (PO_4) are presented in Table 3 in the form of mean ± standard deviation. Overall, the water quality was within the acceptable range for Pacific white shrimp, *Litopenaeus vannamei*.

3.2 | Growth performance

Based on the growth performance and survival of Pacific white shrimp, *L. Vannamei*, fed with the experimental diets presented in Table 4, the final biomass (Bio), final mean weight (FMW), thermal growth efficient (TGC), percentage weight gain (PWG), and average daily growth (ADG) were significantly affected by the dietary treatments ($o < 0.0001$). The highest FMW, PWG, TGC, and ADG were obtained in the group of shrimp fed with 5 and 10% of DDGS compared with the control and D15 treatment ($p < 0.0001$). Meanwhile, the

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

		Analysis results	
Parameters	Unit	Morning	Afternoon
Dissolved oxygen	mg L^{-1}	5.71 ± 0.28	5.75 ± 0.33
Temperature	°C	29.07 ± 0.34	29.26 ± 1.11
Salinity	‰	34.44 ± 0.78	34.10 ± 0.98
pH		7.73 ± 0.12	7.84 ± 0.21
Ammonia (NH_3-N)	mg L^{-1}	0.208 ± 0.062	
Nitrite (NO_2-N)	mg L^{-1}	0.062 ± 0.004	
Nitrate (NO_3-N)	mg L^{-1}	5.390 ± 0.695	
Phosphate (PO_4)	mg L^{-1}	0.084 ± 0.035	

dietary treatments did not significantly affect the survival rate (SR) of shrimp during 90-day culture period ($p = 0.9689$).

3.3 | Body composition analysis

The whole body composition, both proximate and AA level on the shrimp fed diets containing various inclusion levels of DDGS, was observed at the end of the growth trial and presented in Table 5. There was no significant difference between the crude fiber and ash content of shrimp. Nonetheless, numerically, we did observe a difference in crude protein and lipid content of the shrimp. Crude protein and lipid increased at 5 and 10% inclusion levels of DDGS but later decreased with increasing inclusion of DDGS up to 15% in shrimp diet.

3.4 | Total hemocyte count and lysozyme activity

The total hemocyte counts (THC) of shrimp at the end of the growth trial were not significantly affected by different inclusion levels of DDGS in their diet (Figure 1). In addition, the dietary treatment also did not significantly affect the lysozyme activity of shrimp after feeding the shrimp for 90 days (Figure 2).

3.5 | Organoleptic evaluation

The results of the overall scores obtained in each case are shown in Table 6. In this case, the values were the average of the evaluations of 15 people. An error of 5% was determined. Statistically, there was no significant difference between the experimental diets on the shrimp's color, aroma, and flavor. However, numerically, the data demonstrated that there is an increase in shrimp color after being fed with diet containing 5%, 10%, and 15% DDGS for 90 days and increase in shrimp flavor on shrimp fed with 10% and 15% DDGS. In terms of texture, the inclusion of 10% and 15% DDGS significantly increases the tenderness of the shrimp.

TABLE 4 Growth performance of pacific white shrimp, *Litopenaeus vannamei* (Mean initial weight 1.06 ± 0.01 g), fed experimental diets for 90 days in commercial ponds. Values represent the mean of 10 replicates.

Diet code	Final biomass (g)	Final mean weight (g)	Survival (%)	PWG[a] (%)	FCR[b]	TGC[c]	ADG[d]
DO	12089.00b	19.861b	84.55	1769.27b	1.245a	0.0669b	0.214b
D5	12282.00a	20.187a	84.50	1799.95a	1.224b	0.0676a	0.217a
D10	12352.00a	20.252a	84.72	1806.08a	1.219b	0.0677a	0.218a
D15	12074.00b	19.848b	84.49	1768.05b	1.246a	0.0670b	0.214b
p-value	<0.0001	<0.0001	0.9689	<0.0001	<0.0001	<0.0001	<0.0001
PSE[e]	80.9602	0.0964	0.6572	9.0783	0.0064	0.0002	0.0011

Note: Results in the same columns with different letters are significantly different ($p < 0.05$) based on analysis of variance followed by Tukey's multiple comparison test.
[a]PWG = Percentage weight gain.
[b]FCR = Feed conversion ratio.
[c]TGC = Thermal growth coefficient.
[d]ADG = Average daily growth in gram per day.
[e]PSE = Pooled standard error.

TABLE 5 Proximate composition and amino acid profile (% dry weight) of the whole body of shrimp *Litopenaeus vannamei* in the dry condition offered experimental diets for 90 days.

			Nutritional composition			
No	Parameter	Unit	D0	D5	D10	D15
Proximate composition analysis[a]						
1	Protein Content	%	42.95	43.69	43.78	43.33
2	Total Fat	%	12.50	14.10	14.50	14.10
3	Water content	%	7.12	8.65	8.07	7.16
4	Ash Content	%	10.56	10.22	10.22	10.10
5	Crude fiber	%	<0.02	<0.02	<0.02	<0.02
Amino acid composition analysis[b]						
1	L-Cysteine	%	2.04	2.52	1.88	2.09
2	L-Methionine	%	1.09	1.38	1.04	1.01
3	L-Serine	%	1.55	1.72	1.38	1.25
4	L-Glutamic acid	%	4.30	4.62	3.78	3.51
5	L-Phenylalanine	%	1.72	2.18	1.72	1.45
6	L-Isoleucine	%	1.46	1.59	1.26	1.23
7	L-Valine	%	1.64	1.77	1.49	1.38
8	L-Alanine	%	2.36	2.62	2.21	2.04
9	L-Arginine	%	2.90	3.43	2.24	2.11
10	Glycine	%	3.02	3.89	3.16	2.88
11	L-Lysine	%	2.03	2.03	1.52	1.55
12	L-Aspartic Acid	%	2.57	2.79	2.35	2.12
13	L-Leucine	%	2.56	2.85	2.23	2.16
14	L-Tyrosine	%	1.27	1.62	1.27	1.10
15	L-Proline	%	2.19	2.39	2.07	1.91
16	L-Threonine	%	1.63	1.78	1.45	1.31
17	L-Histidine	%	0.81	0.96	0.78	0.69
18	L-Tryptophan	%	0.23	0.27	0.26	0.23

[a]Analyzed at the Feed and Nutrition Laboratory, Faculty of animal and agricultural sciences, Diponegoro University (Semarang, Indonesia).
[b]Analyzed at the Saraswanti Indo Genetech laboratory (Bogor, West Java, Indonesia).

4 | DISCUSSION

In this research, the inclusion of 5% and 10% corn DDGS was able to improve the final mean weight (FMW), percentage weight gain (PWG), and thermal growth coefficient (TGC) of Pacific white shrimp compared with the group of shrimp fed without DDGS. Under the reported condition, there was a significant decrease on growth performance of shrimp when the inclusion level of DDGS was increased up to 15% compared with the performance of shrimp fed with 5% and 10% DDGS but with no difference compared with the control treatment. Using identical feed formulation, our previous results done in laboratory tank conditions using two-stage growth trials showed no significant differences in terms of growth performance of shrimp among the dietary treatments when compared with the control treatment (Novriadi, Suwendi, & Tan, 2022). Rhodes et al. (2015) reported that the use of lipid-extracted DDGS up to 20% is recommended to substitute the use of SBM in the shrimp *L. vannamei* diet. In addition, the study by Gyan et al. (2022) even suggests

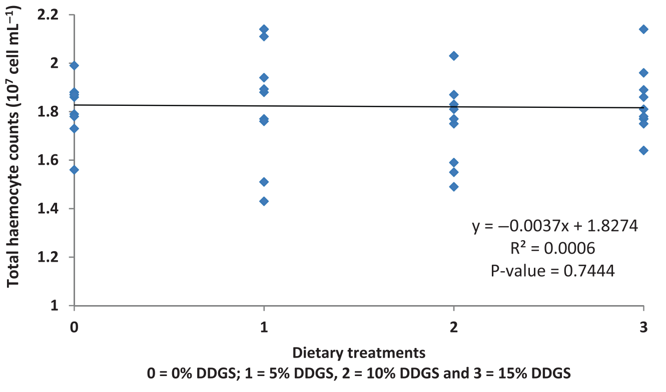

FIGURE 1 Relationship between corn distiller's dried grain with solubles (DDGS) inclusion level in the diet and total hemocyte count (THC) of Pacific white shrimp, *Litopenaeus Vannamei* (10^7 cell mL^{-1}), at the end of the growth trial. Values represent the mean of 10 replicates. Dietary treatments 0 = control; 1 = 5% corn DDGS; 2 = 10% corn DDGS; and 3 = 15% corn DDGS (*R*-value = 0.0006 and *p*-value = 0.7444).

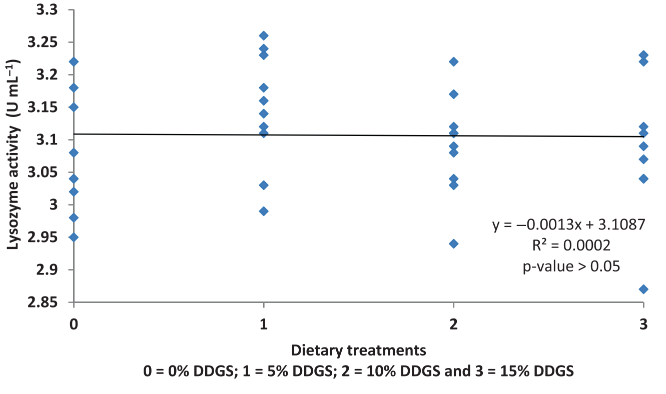

FIGURE 2 Relationship between corn distiller's dried grain with solubles (DDGS) inclusion level in the diet and lysozyme activity of Pacific white shrimp, *Litopenaeus Vannamei* (U mL^{-1}), at the end of the growth trial. Values represent the mean of 10 replicates. Dietary treatments 0 = control; 1 = 5% corn DDGS; 2 = 10% corn DDGS; and 3 = 15% corn DDGS (*R*-value = 0.0002 and *p*-value >0.05).

TABLE 6 Results of organoleptic assessment to the shrimp fed with dietary treatments ($n = 15$).

	Parameters			
Treatments	Color	Aroma	Flavor	Texture
DO	3.67	3.33	3.60	3.13
D5	3.73	2.87	3.47	3.67
D10	3.73	3.20	3.93	3.87
D15	4.00	3.60	4.07	4.00
p-value	0.5711	0.1707	0.1812	0.0322
PSE	0.2804	0.8051	0.6801	0.4000

that replacing fish meal (FM) as the most preferable protein source for aquafeed, with 8% DDGS still able to enhance the growth performance of shrimp *L. vannamei*. Gyan et al. (2022) further explained that the changes in AA content after supplementing the diet with lysine, methionine, arginine, and threonine may promote better growth in shrimp fed with DDGS. Considering this other than formulating feeds that meet the specific nutrient requirement of shrimp, the lack of ANFs and the presence of high fiber content in DDGS to increase the energy of diet could explain the better growth obtained in long-term culture period and out-door situation compared with the controlled environment.

In this research, the dietary treatment also caused a significant effect to the feed conversion ratio (FCR) but not with the SR of shrimp. The FCR of shrimp using predetermined feeding regime tended to be more efficient in the group of shrimp fed with 5 and 10% DDGS compared with the shrimp fed with control diet and 15% DDGS. Previous research in tank trials showed that the inclusion of 5%–15% corn DDGS did not cause any significant effect to the FCR (Novriadi, Suwendi, & Tan, 2022). The increase in FCR as the inclusion of DDGS increased to 15% could be because of overfeeding, because the final weights of shrimp fed with 15% DDGS are reduced. Looking at the survival, the percentage survival obtained in this study was similar with studies using controlled environment (Novriadi, Roigé, & Segarra, 2022; Yaemsooksawat et al., 2009). Because all measured water quality parameters in this research were still within the acceptable range for growth and health condition of shrimp, the culture environment supported the survivability of shrimp during the culture period.

Whole body analysis of shrimp *L. vannamei* expressed in percent dry matter basis showed that crude fiber and ash content were not affected when DDGS is used to replace SBM in shrimp diet, and this was consistent with the previous study using clear water in aquaria and round tanks (Novriadi, Suwendi, & Tan, 2022). In addition, the study by Gyan et al. (2021) also showed that there is no significant effect to the ash content as the inclusion levels of DDGS increased to replace the use of FM. In the present study, the crude protein and lipid content were affected in shrimp fed with 5% and 10% inclusion levels of DDGS but later decreased with increasing inclusion level of DDGS up to 15% in shrimp diet. Looking at the comparable value of protein and lipid content in the formulated diet, the nutrient availability is not likely the cause to alter the nutrient composition in the whole body of shrimp. The decreased protein and lipid content in shrimp fed with higher levels of DDGS could be because of the reduction in feed intake and growth in the group of shrimp fed with 15% DDGS that showed a decreasing trend.

It has been proposed that the use of DGGS as an alternative ingredient could affect the metabolism, immune function, and intestinal health of the aquatic organisms (Gyan et al., 2021; Mostafizur Rahman et al., 2015; Zhu et al., 2022). DDGS, in which 5.3% of the protein content is contributed by yeast protein, containing B-complex and ß-glucan (Webster & Thompson, 2015), demonstrated a significant potential to improve the immune response in shrimp and fish (Ceseña et al., 2021; Sakai, 1999; Sohn et al., 2000; Zhang et al., 2018). Various studies have shown that β-glucans, as one of the substances contained in the yeast protein, were also able to induce the activation of hemocytes in crustaceans (Ceseña et al., 2021; Gyan et al., 2021; Perveen et al., 2021). Once β-glucans are detected, hemocytes are activated and neutralize or eliminate infective agents through phagocytosis and encapsulation. The detection of β-glucans can also induce the release of bioactive molecules that assist in phagocytosis, such as microbial proteins (e.g. lectins), agglutinins, hydrolytic enzymes, and antimicrobial peptides (Söderhäll & Cerenius, 1998). Furthermore, yeast could also improve the lysozyme activity in crustacean to degrade the Gram-negative bacteria cell wall, allowing their recognition by phagocytic cells (Aguirre-Guzman et al., 2009; Xiong et al., 2018). In this research, total hemocyte count (THC) and lysozyme activity of shrimp were not affected by various inclusion levels of DDGS to partially replace the inclusion of SBM in formulated diet. The study by Novriadi, Wahyudi, et al. (2022)) revealed that even the use of corn fermented protein (CFP) as a novel ingredient generated from further processing technique of DDGS was also not able to enhance the THC (Figure 1) and lysozyme activity (Figure 2) of shrimp *L. vannamei*. Because there are limited data on the effect of partial replacement of SBM with various inclusion levels of DDGS to the THC and lysozyme activity in shrimp, further study is needed to identify the effect of DDGS to modulate the nonspecific immune system of shrimp in different growth stage both in an out-door pond and controlled environment.

In global market, nutritional and organoleptic qualities (taste, smell, texture, and appearance) can be an important differentiator when it comes to defining consumer choice (Chemel et al., 2020). The results of organoleptic analysis in this research showed that there were no significant differences in terms of shrimp color, aroma, and flavor. Because the analysis involved a group of trained and experienced people, the results showed that there is no effect on the quality of the shrimp as the protein source changed from SBM to DDGS. Furthermore, the results of this research showed that there is a significant increase in terms of shrimp tenderness in the group of shrimp fed with 10% and 15% DDGS compared with the control treatment. Changes in tenderness might be affected as the impact of modification in shrimp feed formulation (Ezquerra Brauer et al., 2003), especially with corn and corn-based products (Shephard et al., 1996). However, proper feed formulation and the use of good quality ingredients could prevent the changes (Mexía-Salazar et al., 2008). The present study provides useful information about some aspects on shrimp quality after feeding the shrimp with DDGS to partially replace the inclusion of SBM using an out-door pond until the shrimp reached consumption size. The results might lead to a design for quantitative sensory analysis in shrimp to reveal the effect of using DDGS to the quality of shrimp.

5 | CONCLUSION

The results from the present study confirm that DDGS can be included up to 15% in the practical diet of Pacific white shrimp *Litopenaeus vannamei* to replace the use of SBM without affecting the growth performance, health, and the product quality. Optimum growth was obtained with the inclusion of 5% and 10% DDGS supplemented with AAs to partially replace the use of SBM. Furthermore, replacing SBM with DDGS did not cause any significant effect to the total hemocyte counts (THC) and lysozyme activity in shrimp. In addition, the inclusion of DDGS up to 15% was still able to maintain the quality of the shrimp, especially for the color, aroma, and flavor characteristics. Results from this study indicate that the texture values of shrimp significantly improve with the inclusion of 10% and 15% DDGS to partially replace SBM. Nonetheless, further studies are needed for a quantitative analysis for detailed sensory observation.

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CONFLICT OF INTEREST STATEMENT

All authors state no conflict of interest except Ronnie Tan who has been employed as a consultant by U.S. Grains Council.

DATA AVAILABILITY STATEMENT

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

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