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GROWTH PERFORMANCE AND HEALTH STATUS RESPONSE OF THE PACIFIC WHITE-LEG SHRIMP *PENAEUS VANNAMEI* BOONE, 1931 (DECAPODA, DENDROBRANCHIATA) FED A COMMERCIAL DIGESTIBILITY ENHANCER IN LOW FISH MEAL DIET WITH PALM KERNEL MEAL REPLACEMENT

BY

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ABSTRACT

The effect of a digestibility enhancer (DE) in a low fish meal diet on the growth and health status of the Pacific whiteleg shrimp, *Penaeus vannamei* was investigated using seven iso-nitrogenous and iso-lipidic (35% protein and 6% lipid) diets. These seven experimental diets were fed to *P. vannamei*, divided into five replicate experimental groups of shrimps assigned per dietary treatment, for 90 days. The feed conversion ratio (FCR) showed significant differences ($p<0.05$) with the diet of 6% palm kernel meal (PKM) + DE delivering the best feed efficiency. Meanwhile, there were no significant differences in the survival rate (SR) found, however, numerically the group of shrimps fed with 2.5% PKM with DE had a higher SR compared to the other dietary treatments. For growth performance, no significant differences in the growth performance of the shrimp among the dietary treatments were observed, including also the date for the final body weight and the percentage weight gain. The body composition of the shrimp showed slight changes in nutrient retention, where those shrimp fed the diet with DE supplementation had a marginal increase in protein content (21.33% compared to 20.53% in the control) and energy content (96.46 kcal/100 g with 9% PKM + DE, versus 89.79

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kcal/100 g in the control). In terms of health criteria, *P. vannamei* appeared to show changes in their status for specific indicators. This was apparent for shrimp fed increasing PKM irrespective of the additive for the total haemocyte counts. There was a progressive reduction in the level of lysozyme in shrimp fed PKM and this effect was accentuated for the PKM treatment receiving DE. The inclusion level of DE as much as 0.2% in feed formulated with graded inclusion levels of PKM, up to 9% to partially reduce the inclusion of fish meal and soybean meal, is still able to maintain the optimal growth and health of the shrimp, at the same time, we noted an increase in production efficiency by reducing feed formulation costs by 1.23-3.87%.

Key words. — *Penaeus vannamei*, palm kernel meal, digestibility enhancer, growth and health response

RÉSUMÉ

L'effet d'un améliorateur de digestibilité (DE) dans un régime alimentaire pauvre en farine de poisson sur la croissance et l'état de santé de la crevette blanche du Pacifique, *Penaeus vannamei*, a été étudié à l'aide de sept régimes alimentaires iso-nitrogéniques et iso-lipidiques (35% de protéines et 6% de lipides). Ces sept régimes expérimentaux ont été administrés à des *P. vannamei*, réparties en cinq groupes expérimentaux de crevettes assignés par traitement alimentaire, pendant 90 jours. Le taux de conversion alimentaire (FCR) a montré des différences significatives ($p<0{,}05$), le régime composé de 6% de farine de palmiste (PKM) + DE offrant la meilleure efficacité alimentaire. Par ailleurs, aucune différence significative n'a été observée en termes de taux de survie (SR). Cependant, numériquement, le groupe de crevettes nourries avec 2,5% de PKM et de DE a présenté un SR plus élevé que les autres groupes soumis à différents régimes alimentaires. En ce qui concerne les performances de croissance, aucune différence significative n'a été observée entre les différents groupes soumis à différents régimes alimentaires, y compris pour le poids final et le pourcentage de gain de poids. La composition corporelle des crevettes a montré de légers changements dans la rétention des nutriments, les crevettes nourries avec un régime enrichi en DE présentant une augmentation marginale de la teneur en protéines (21,33% contre 20,53% dans le groupe témoin) et de la teneur énergétique (96,46 Kcal/100 g avec 9% de PKM+DE, contre 89,79 Kcal/100 g dans le groupe témoin). En termes de critères sanitaires, *P. vannamei* a semblé présenter des changements dans son état pour certains indicateurs spécifiques. Ceci a été particulièrement évident chez les crevettes nourries avec des quantités croissantes de PKM, indépendamment de l'additif utilisé pour le nombre total d'hémocytes. On a observé une réduction progressive du taux de lysozyme chez les crevettes nourries avec du PKM, et cet effet était accentué chez celles ayant reçu un traitement au PKM associé à DE. L'ajout de DE à hauteur de 0,2% dans les aliments formulés avec des niveaux d'ajout graduels de PKM, jusqu'à 9% afin de réduire partiellement l'ajout de farine de poisson et de farine de soja, permet toujours de maintenir une croissance et une santé optimales des crevettes ; en même temps il est constaté une augmentation de l'efficacité de la production grâce à une réduction des coûts de formulation des aliments de 1,23 à 3,87%.

Mots clés. — *Penaeus vannamei*, farine de palmiste, améliorateur de digestibilité, réponse de croissance et de santé

INTRODUCTION

Currently, the shrimp industry is experiencing challenges with rising feed prices driven by increasing prices of raw materials, especially fish meal (Hernández et al., 2004; Amaya et al., 2007; Nunes et al., 2022). This encourages searching for alternative protein sources, such as soybean meal (SBM), corn meal and corn by-products, as well as cottonseed meal, to replace the expensive ingredients, such as fish meal (FM) (Nunes et al., 2022; Novriadi et al., 2023a). Indeed, a critical evaluation process is needed prior to including those alternative ingredients in the diet formulation, focusing on the nutritional characteristics, palatability, digestibility, impact on the immune system, and on the health condition of the shrimp, the actual functionality of the replacing feed component and, in general, the quality of all the ingredients (Glencross, 2020). Apart from the alternatives mentioned above, palm kernel meal (PKM) as a common by-product from the palm kernel oil extraction process, also has the potential to be used in the development of aquafeed (Ng, 2003; Sangavi & Betsy, 2020), especially in countries such as Indonesia and Malaysia, which are the largest palm oil-producing countries "in the world", producing 85-90% of the world's palm oil production (Ibrahim, 2013; Sehgal & Sharma, 2021; Masitah et al., 2023).

An increase in the production of palm kernel meal (PKM) and cake (PKC) has already led to research in order to establish the optimum inclusion levels of PKM and PKC in diets for broiler chickens (Sundu et al., 2006; Azizi et al., 2021), growing pigs (Agunbiade et al., 1999; Kini et al., 2020), and ruminants (Umunna et al., 1980; Abdeltawab & Khattab, 2018). In recent years, PKM and PKC have also begun to be used in the development of diets for fish and shrimp (Shamsuddin, 2017; da Silva et al., 2020; Mazón Paredes et al., 2020; Paredes et al., 2020; Shamsuddin et al., 2021). The use of PKM is a noteworthy option due to its protein content, ranging from 12-21% protein (Sundu et al., 2006), and a substantial 36% of essential amino acids (Safi et al., 2022). The nutritional profile does position PKM as a valuable substitute for more expensive ingredients (da Silva et al., 2020; Shamsuddin et al., 2021). The main challenge of using palm kernel meal are the large amounts of cell wall constituents (Ng & Chen, 2002; Ng et al., 2002), the lack of carbohydrates (Thongprajukaew et al., 2013), and the lower digestibility (Thongprajukaew et al., 2015) that could inhibit the wider use of PKM in the diet formulation, in this case specifically for shrimp culture.

Functional additives, such as enzymes and plant extracts, can be applied to mitigate the challenges associated with the inclusion of PKM and PKC in the feed (Siti-Noritaac et al., 2015; Wattanakul et al., 2021). In addition, the inclusion of freeze-dried β-mannanase at a level of 10 g/kg could improve the apparent nutrient digestibility of crude protein, ash, and fibre of the diet formulated with 400 g/kg of PKC and fed to red tilapia (Siti-Noritaac et al., 2015). Moreover, the physical modifications of PKM by using water soaking and microwave irradiation methods can improve the in vitro carbohydrate digestibility in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) and the striped snakehead, *Channa striata* (Bloch, 1793) (cf. Thongprajukaew et al., 2013; 2015). However, until recently, there has been only limited research regarding digestibility enhancers produced from a mixture of plant extracts and amino acids to improve the performance of feed formulated using PKM. Therefore, this research aimed to evaluate the use of a commercial digestibility enhancer, which is a mixture of essential oils of oregano and lemongrass, and also a mixture of the amino acids lysine and threonine (DE, Economix, Techna France Nutrition, France) on the growth rate and health condition of the Pacific whiteleg shrimp, *Penaeus vannamei*. We conducted a comprehensive study, observing the growth performance, total haemocyte counts (THC) and lysozyme activity of shrimp reared in so-called "hapa nets" (a cage-like rectangular or square net in which small fish or crustaceans can be kept in outdoor aqua- or mari-culture) installed within an outdoor pond, like the commercial production conditions, until the shrimp reached their harvestable size.

MATERIAL AND METHODS

Experimental diets

The experimental diets were formulated to include increasing levels of palm kernel meal (PKM) in order to reduce the dietary inclusion levels of fish meal (FM) and soybean meal (SBM). Then, three diets were supplemented with a digestibility enhancer (DE, Economix, Techna France Nutrition), and another three diets did not receive the DE supplementation. In total, seven iso-nitrogenous and iso-lipidic (35% protein and 6% lipid) diets were prepared, including a control diet using 10% FM and 39.94% SBM without PKM and DE. Three experimental feeds were designed to reduce FM and SBM levels, and were substituted with 2.5, 6 and 9% inclusion level of PKM, respectively. The diets were further supplemented with DE, and labelled 2.5, 6 and 9% PKM + DE. In contrast, the following three research feeds were formulated with 2.5, 6 and 9% PKM without DE supplementation and labelled 2.5, 6 and 9% PKM (table I). All dry ingredients were carefully weighed and mixed in a paddle mixer (Marion Mixers, Marion, IA, U.S.A.) in a 100 kg batch, followed by grinding to a particle size of $<200\ \mu$m using a disk mill (Jinan Shengrun, P.R. China). Fish oil was then added and mixed constantly. A twin extruder (Jinan Shengrun) was used to extrude the feed through a 2 mm die (i.e., a small nozzle) at a temperature gradient of 62, 80 and 110°C in three zones of the extruder barrel and the die head, respectively. All diets were oven-dried at 50-70°C in a pulse bed dryer (Jinan Shengrun). All finished diets were bagged and stored in a temperature-controlled room until further use. The proximate and amino acid profiles of the diets are summarized in table II.

Experimental shrimp and feeding programme

Post larvae (PL) of Pacific whiteleg shrimp, *Penaeus vannamei* were obtained from PT. Windu Alam Sentosa (Rembang, Central Java, Indonesia) and then trans-

TABLE I

Composition (% as is) of diets consisting of several inclusion levels of digestibility enhancer (DE; Economix, Techna France Nutrition, France) in commercial diet formulations by utilizing palm kernel meal (PKM) and fed to *Penaeus vannamei* Boone, 1931 for 90 days

Composition	Control	2.5% PKM + DE	6% PKM + DE	9% PKM + DE	2.5% PKM	6% PKM	9% PKM
Fishmeal[1]	10.00	10.00	9.00	8.00	10.00	9.00	8.00
Soybean meal[1]	39.94	38.71	38.38	38.33	38.71	38.38	38.33
Palm kernel meal[2]	0.00	2.50	6.00	9.00	2.50	6.00	9.00
Wheat flour[3]	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Corn starch[2]	14.05	12.84	11.25	9.84	13.04	11.45	10.04
Poultry meal[1]	12.00	12.00	11.50	11.00	12.00	11.50	11.00
Digestibility enhancer[4]	0.00	0.20	0.20	0.20	0.00	0.00	0.00
Fish oil[1]	3.61	3.41	3.24	3.11	3.41	3.24	3.11
MCP[1]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Lecithin[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cholesterol[5]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mineral premix[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[7]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methionine[5]	0.36	0.34	0.36	0.38	0.34	0.36	0.38
Lysine[5]	0.33	0.29	0.36	0.43	0.29	0.36	0.43
Vit C (Aner C)[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Formulation cost (IDR)/kg[8]	13 204	13 042	12 840	12 693	12 907	12 705	12 558

TABLE I
(Continued)

Composition	Control	2.5% PKM + DE	6% PKM + DE	9% PKM + DE	2.5% PKM	6% PKM	9% PKM
Price gap[9] (%)		1.23	−2.76	−3.87	−2.25	−3.78	−4.89

[1] PT FKS Multi Agro, Tbk. Jakarta, Indonesia.
[2] PT Rajawali Mitra Pakanindo, Banten, Indonesia.
[3] PT Pundi Kencana, Cilegon, Banten, Indonesia.
[4] Economix (E, Techna France Nutrition, France).
[5] Fenanza (South Jakarta, Jakarta, Indonesia).
[6] Trace mineral premix (g/100 g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.550; ferrous sulfate, 2.000; magnesium sulfate anhydrous, 13.862; manganese sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; alpha-cellulose, 69.664.
[7] 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 (5×10^5 IU/g), 0.32; vitamin D3 (10^6 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 856.81.
[8] IDR, Indonesian Rupiah.
[9] $\text{Price gap } \% = \frac{\text{Formualted price of control diet} - \text{formulated price of experimental diet}}{\text{Formulated price of control diet}} \times 100$

TABLE II

Proximate and amino acid (AA) composition (% as is, dry matter basis) of experimental diets utilized in the feeding trial of *Penaeus vannamei* Boone, 1931

Composition	Unit	Control	2.5% PKM + DE	6% PKM + DE	9% PKM + DE	2.5% PKM	6% PKM	9% PKM
Ash content	%	8.84	8.68	8.44	8.24	8.35	8.69	8.37
Total fat	%	59.01	64.08	66.69	56.3	59.94	63.63	66.87
Moisture content	%	9.31	9.76	8.22	8.97	9.37	8.69	8.57
Protein content	%	35.75	35.27	35.03	34.08	34.89	34.78	34.22
Amino-acid profile								
L-serine	%	1.92	2.01	2.14	1.88	1.90	1.89	1.98
L-Glutamic acid	%	4.53	4.72	4.92	4.88	4.85	4.94	4.92
L-Phenylalanine	%	2.12	1.75	1.86	1.92	1.92	1.80	2.11
L-Isoleucine	%	1.26	0.98	1.12	1.23	1.35	1.37	1.36
L-Valine	%	1.49	1.21	1.65	1.55	1.59	1.61	1.62
L-Alanine	%	1.54	1.54	1.73	1.55	1.63	1.73	1.60
L-Arginine	%	2.92	2.26	2.69	2.94	2.61	2.64	3.05
Glycine	%	1.93	1.86	1.86	1.92	1.82	1.91	1.93
L-Lysine	%	1.79	1.80	1.83	1.80	1.79	1.79	1.79
L-Aspartic acid	%	2.48	2.46	2.62	2.61	2.62	2.63	2.61
L-Leucine	%	2.51	2.25	2.64	2.65	2.59	2.64	2.64
L-Tyrosine	%	1.19	0.96	1.03	1.08	0.97	0.98	1.13
L-Proline	%	2.01	2.02	2.04	2.11	2.10	2.20	2.13
L-Threonine	%	1.53	1.54	1.55	1.56	1.50	1.50	1.51
L-Histidine	%	0.92	1.15	1.23	1.05	0.85	0.83	0.96
L-Tryptophan	%	0.25	0.23	0.26	0.23	0.23	0.24	0.24
L-Cystine	%	1.55	2.35	2.37	2.39	1.73	1.93	1.87
L-Methionine	%	0.47	0.48	0.46	0.46	0.43	0.45	0.44

* Analysis conducted by the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia (www.siglaboratory.com).

TABLE III

Water quality analysis during the 90 days of observation in the culture pond in which the feeding experiments with *Penaeus vannamei* Boone, 1931 were performed

Parameters	Unit	Results of the analyses			
		5.30 AM	11.59 AM	5.00 PM	9.00 PM
Physical parameters					
Dissolved oxygen	mg/l	6.21 ± 0.22	8.23 ± 0.67	6.62 ± 0.35	5.86 ± 0.54
Temperature	°C	27.33 ± 0.51	29.80 ± 0.97	29.71 ± 1.34	28.37 ± 0.79
Salinity	g/l	24.63 ± 6.65	26.37 ± 3.90	26.51 ± 3.85	26.52 ± 3.93
pH	–	8.18 ± 0.16	8.57 ± 0.18	8.73 ± 0.22	8.54 ± 0.26
Chemical parameters (analysis was performed once a week)					
Ammonium (NH_4)	mg/l		0.08 ± 0.10		
Nitrate-nitrogen (NO_3-N)	mg/l		0.02 ± 0.03		
Nitrite-nitrogen (NO_2-N)	mg/l		0.03 ± 0.03		
Phosphate (PO_4)	mg/l	0.08 ± 0.04			
Biological parameters (analysis was performed once a week)					
Total bacteria	(10^3 CFU/ml)		8.24 ± 4.44		
Total *Vibrio*	(10^3 CFU/ml)		1.72 ± 1.03		

ported to the nursery facility at the Installation for Marine and Fisheries Field Practices, Jakarta Technical University of Fisheries located in Serang, Banten, Indonesia. The shrimp were acclimatized to the culture environment and fed with commercial feed (CJ feed code SA, 34-36% crude protein) for one week. The acclimatized shrimp (0.70 ± 0.01 g initial mean weight) were then randomly distributed into 35 nets with the size of $2 \times 2 \times 1$ m. Five replicate groups of shrimps were administered different types of experimental diets using a 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. The amount of feed given to shrimp each day during the 90-day experimental period was based on historical data on shrimp growth and a feed conversion ratio of 1.5. Changes in the feed quantity ratio are then made if mortality occurs, or if there are changes in the water quality conditions of the culture media. Water quality observations are then carried out for physical parameters, which include pH, salinity, temperature, and dissolved oxygen; then chemical parameters, which include ammonium (NH_4), nitrate-nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), and phosphate (PO_4); as well as biological parameters for total bacteria and total *Vibrio* spp. count. All water quality data are summarized in table III.

Sampling

At the end of the feeding trial, the shrimp in each hapa net were counted, group-weighed to calculate the final biomass, final weight, percentage weight gain

(PWG), feed conversion ratio (FCR), percentage survival (SR), and the thermal unit growth coefficient (TGC) as follows:

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

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

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

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

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

Analysis of proximate and amino acid profile of the shrimp

At harvest time, or on the 90th day of the observation period, twenty-five shrimp per treatment, or five shrimp from each hapa net, were randomly sampled and stored at −80°C for body composition analysis. Prior to proximate, energy, and amino acid analyses, dried whole shrimp were rigorously blended and chopped in a mixer according to methods described by Helrich (1990). The proximate composition and amino acid profile of the whole shrimp body were analysed at the Saraswati Indo Genetech Laboratory (Bogor, West Java, Indonesia).

Total haemocyte count

At harvest time, haemolymph was sampled from three intermoult shrimp per hapa net, or fifteen shrimp per dietary treatment, and the total haemocyte count was determined. Haemolymph (100 μl) of individual shrimp was withdrawn from the pleopod base of the second abdominal somite with a sterile 1-ml syringe (25 G × 13 mm needle). Before haemolymph extraction, the syringe was loaded with a pre-cooled (4°C) solution (10%-EDTA, Na2) used as an anticoagulant. The haemolymph with an anticoagulant solution was diluted in 150 μl of formaldehyde (4%), and then 20 μl was placed on a haemocytometer (so-called Neubauer-chamber) to determine THC using an optical microscope (Olympus DP72, Japan).

Lysozyme activity analysis

At harvest time, lysozyme activity was measured using a lysozyme detection kit (Sigma-Aldrich, Saint Louis, MO, U.S.A., Cat. no. LY0100) following the manufacturer's instructions. The lysis of the cells of *Micrococcus lysodeikticus* (currently preferably referred to as *Micrococcus luteus* (Schroeter, 1872) Cohn, 1872) defined the results of lysozyme activity. The reactions were conducted at 25°C, and absorbance at 450 nm was measured on the ultraviolet/visible spectrophotometer (Lambda XLS, Perkin Elmer).

$$\text{Lysozyme activity}\left(\frac{\text{Units}}{\text{ml}}\right) = \frac{(\Delta A_{450}/\,\text{min Test} - \Delta A_{450}/\,\text{min Blank})(\text{df})}{(0.001)(0.03)}$$

in which:

df = dilution factor
0.001 = ΔA_{450} as per the unit definition
0.03 = Volume (in ml) of enzyme solution

Statistical analysis

The Shapiro-Wilk test assessed the normality of the data distribution, and the Brown-Forsythe's tests tested the homogeneity of variance before data analysis. Growth parameters, total haemocyte counts, and lysozyme activity were analysed using regression and One-way analysis of variance (ANOVA) to determine significant differences among treatments, followed by Tukey's multiple comparison tests to determine the difference between treatment means among the treatments. All statistical analyses were conducted using the SAS system (V9.4. SAS Institute, Cary, NC, U.S.A.).

RESULTS

Nutritional profile of experimental feed

The data in table II show that even though the experimental feed for *Penaeus vannamei* was designed to be iso-nitrogenous and iso-lipidic, the addition of palm kernel meal (PKM) and digestibility enhancer (DE, Economix, Techna) into the diet had an effect of changes in the protein and fat levels in the feed. Control feed formulated without PKM had the highest protein level compared to other dietary treatments. The inclusion of PKM has a negative impact on the protein level, where the increasing use of PKM leads to a decreasing level of protein (%). Adding a digestibility enhancer contained with a mixture of amino acids, especially lysine and threonine, increased the feed protein levels to be higher compared to the control diet. In detail, feeds that were supplemented in combination with PKM had better levels of lysine, methionine, and threonine than feed formulated with PKM without the addition of DE (table II). For total fat, the addition of PKM increased the total fat in feed compared to the control diet formulated without PKM and digestibility enhancer (DE) (table II).

TABLE IV
Growth performance of the Pacific whiteleg shrimp, *Penaeus vannamei* Boone, 1931 of mean initial weight 0.70 ± 0.01 g, fed experimental diets for 90 days

Treatment	FBW (g)	FCR	TGC	PWG (%)	SR (%)
Control	19.30	1.65^{ab}	0.7161	2656.74	82.20
2.5% PKM + DE	19.52	1.55^{ab}	0.7202	2688.46	92.17
6% PKM + DE	20.57	1.38^{b}	0.7389	2839.00	87.96
9% PKM + DE	19.40	1.57^{ab}	0.7178	2670.92	91.40
2.5% PKM	18.78	1.74^{a}	0.7063	2582.34	87.20
6% PKM	18.69	1.69^{a}	0.7036	2569.69	89.73
9% PKM	18.66	1.60^{ab}	0.7041	2566.43	88.20
P-value	0.1285	0.0051	0.1314	0.1285	0.2445
RSE	1.0374	0.1229	0.0193	148.1955	6.1849

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. FBW, final body weight; FCR, feed conversion ratio; TGC, thermal growth coefficient; PWG, percentage weight gain; SR, survival rate; RSE, residual standard error.

Water quality

In general, biological parameters are within acceptable conditions or values to support the growth of the shrimp, *P. vannamei*. For physical parameters, the average value from four measurements every day during the 90-day observation period, the dissolved oxygen, temperature, salinity and pH are in the range of 6.55 ± 0.17 mg/l, 28.72 ± 0.29°C, 26.11 ± 1.19 g/l and 8.51 ± 0.04, respectively. For chemical parameters, there were levels of ammonium (NH_4), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and phosphate (PO_4) in the ranges of 0.08 ± 0.10, 0.02 ± 0.03, 0.03 ± 0.03 and 0.08 ± 0.04 mg/l, respectively. Meanwhile, for biological parameters, total bacteria during the 90 days of the culture period were in the range of $(8.24 \pm 4.44) \times 10^3$ CFU/ml and total *Vibrio* spp. were in the range of $(1.72 \pm 1.03) \times 10^3$ CFU/ml.

Growth performance

The feed conversion ratio (FCR) was significantly affected by the addition of DE ($p<0.05$). The 6% PKM + DE showed significantly lower FCR than the 2.5% and 6% PKM diet without DE. Meanwhile, the treatments did not significantly affect the final body weight (FBW), thermal growth coefficient (TGC), percentage weight gain (PWG), and survival rate (SR) of the shrimp (table IV).

Proximate composition of the whole shrimp

Table V shows the nutritional profile of the Pacific whiteleg shrimp, *P. vannamei* at the end of the experiment. The addition of DE increased feed protein retention

TABLE V

Nutritional profile of the shrimp, *Penaeus vannamei* Boone, 1931, at the end of the growth trial (i.e., after 90 days)

No	Parameter	Unit	Control	2.5% PKM + DE	6% PKM + DE	9% PKM + DE	2.5% PKM	6% PKM	9% PKM
1	Protein content	%	20.53	20.88	21.25	21.33	20.46	20.41	20.43
2	Ash content	%	1.74	1.64	1.69	1.70	1.65	1.58	1.62
3	Total fat	%	0.73	0.94	0.86	1.26	0.87	0.84	0.46
4	Moisture content	%	76.73	76.13	76.21	75.76	76.33	75.25	76.22
5	Total calories	kcal/100	89.79	92.64	92.22	96.46	92.98	92.90	90.96
6	Carbohydrate	%	0.48	0.62	0.51	0.46	0.59	0.58	0.53
	Amino-acid profile								
7	L-Serine	%	0.88	0.75	0.67	0.76	0.76	0.71	0.71
8	L-Glutamic acid	%	2.64	3.01	2.66	2.86	2.95	2.12	2.67
9	L-Phenylalanine	%	1.11	1.05	0.88	1.13	1.14	0.75	0.93
10	L-Isoleucine	%	0.59	0.78	0.75	0.78	0.77	0.47	0.79
11	L-Valine	%	0.68	0.85	0.83	0.85	0.83	0.55	0.83
12	L-Alanine	%	1.34	1.41	1.42	1.34	1.41	1.00	1.34
13	L-Arginine	%	1.64	1.51	1.15	1.43	1.41	1.26	1.34
14	Glycine	%	1.37	1.27	1.30	1.29	1.25	0.99	1.32
15	L-Lysine	%	1.07	1.18	1.17	1.12	1.18	1.01	1.13
16	L-Aspartic acid	%	1.53	1.66	1.48	1.54	1.61	1.23	1.46
17	L-Leucine	%	1.40	1.42	1.55	1.41	1.41	1.13	1.41
18	L-Tyrosine	%	0.86	0.83	0.62	0.87	0.88	0.59	0.67
19	L-Proline	%	1.25	1.23	1.14	1.26	1.14	1.06	1.21
20	L-Threonine	%	0.88	0.85	0.72	0.90	0.85	0.67	0.82
21	L-Histidine	%	0.69	0.69	0.43	0.77	0.69	0.49	0.71
22	L-Tryptophan	%	0.14	0.15	0.16	0.14	0.14	0.13	0.15
23	L-Cystine	%	1.68	1.66	1.89	1.96	1.66	2.49	2.21
24	L-Methionine	%	0.23	0.29	0.35	0.32	0.33	0.29	0.33

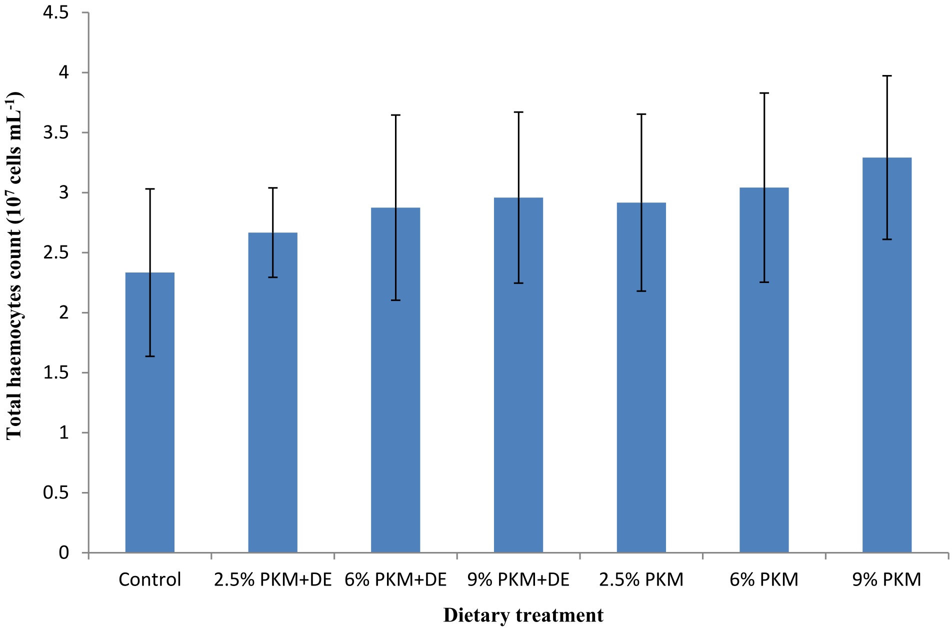

Fig. 1. Value of total haemocyte count (10^7 cells/ml) of the Pacific white shrimp, *Penaeus vannamei* Boone, 1931, after being fed with experimental diets formulated with several inclusion levels of palm kernel meal with or without an additional digestibility enhancer (DE, Economix, Techna) for 90 days cultured in an outdoor pond. Values represent the mean of five replicates ($p>0.05$).

in the shrimp's body with a protein level in the range of 20.88-21.33% and was better than that of the control (20.53%) and higher than the protein retention in the shrimp group that did not receive DE (20.41-20.46%). Better fat content retention was also shown by the shrimp group that received DE supplementation (0.86-1.26%) compared to the control (0.73%) and the shrimp group without DE (0.46-0.87%). Higher protein levels also had a positive impact on higher total calorie content in the shrimp group that received DE (92.22-96.46 kcal/100 g) compared to controls (89.79 kcal/100 g) and shrimp without DE supplementation (90.96-92.98 kcal/100 g). Interestingly, the fat content and total calories of 2.5 and 6% PKM are higher than the control. Adding DE increased the retention of lysine, methionine, and threonine in the shrimp body compared to the control for amino acids.

Total haemocyte count

From the data presented in fig. 1, the value of total haemocyte count (THC, 10^7 cells/ml) of the Pacific whiteleg shrimp *P. vannamei* after being fed with several inclusion levels of DE, did not show any significant difference ($p>0.05$). However, numerically, the value trend showed that the THC value for shrimp fed with PKM

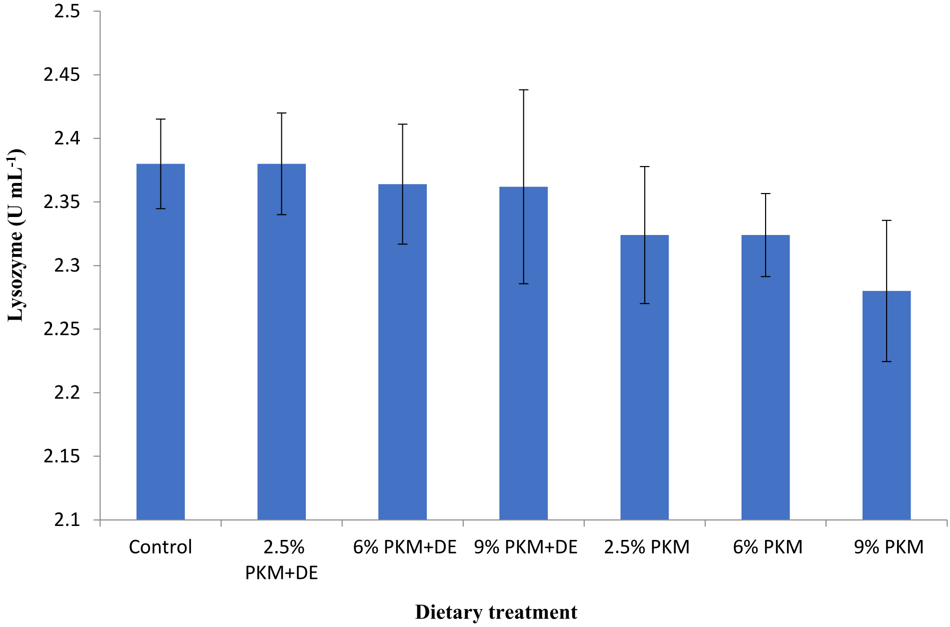

Fig. 2. Value of lysozyme activity (U/ml) in the Pacific white shrimp, *Penaeus vannamei* Boone, 1931, after being fed with experimental diets formulated with incremental inclusion levels of palm kernel meal with or without an additional digestibility enhancer (DE, Economix, Techna) for 90 days cultured in an outdoor pond. Values represent the mean of five replicates ($P > 0.05$).

is higher than in the control treatment. In general, the data suggest that higher inclusion levels of PKM provide higher THC values for both shrimp fed with PKM and those supplemented with or without DE.

Lysozyme activity analysis

The data presented in fig. 2 show that the value of lysozyme activity (U/ml) of the Pacific whiteleg shrimp *P. vannamei* generally does not provide significant differences for all dietary treatments ($p > 0.05$). However, numerically, shrimp-fed control treatment had the highest lysozyme activity compared to other treatments. For feed formulated using PKM, it shows that the greater the inclusion levels of PKM, the lower the lysozyme activity value. The addition of DE to the PKM feed did not have a positive impact on lysozyme activity analysis.

DISCUSSION

Incorporating digestibility enhancers or additives has many positive influences on improving feed performance, especially when trying to substitute premium raw materials, such as fish meal (FM) and soybean meal (SBM), with economical raw materials or when culturing the aquatic organisms under suboptimal environmental conditions (Hossain et al., 2018; Hodar et al., 2020; Magouz et al., 2020; Sajina et al., 2021). In this research on the Pacific whiteleg shrimp, *Penaeus vannamei*, dietary DE significantly increased the feed efficiency, especially in the group of shrimps fed 6% PKM + DE, which had the lowest FCR values compared to all treatments including the control diet. Although, in general, the addition of DE did not provide a significant difference in growth parameters due to variations in the weight of the shrimp at the end of the growth trial, there was a tendency toward better growth values in the group of shrimps fed with several inclusion levels of PKM supplemented with DE compared to the control and even better compared to the shrimp group fed PKM without DE (table IV). This research uses a combination of the essential oils of oregano and lemongrass and a mixture of the amino acids lysine and threonine as a digestibility enhancer. Essential oils of oregano and lemongrass have been known to have an antimicrobial activity, thus inhibiting the growth of pathogenic bacteria (Choi et al., 2012; Moore-Neibel et al., 2012; Torres Neto et al., 2022), and promoting the growth of beneficial gut microorganisms for better nutrient digestion and absorption (Bendini et al., 2002). Meanwhile, the presence of lysine and threonine can promote the nutritional digestion and absorption capacity in aquatic organisms (Cheng et al., 2003; Palavesam et al., 2008; Li et al., 2014; Dong et al., 2022). Therefore, the combination of essential oils and amino acids as digestibility enhancers used in this study could provide similar growth performance between shrimp fed with PKM supplemented with DE and the shrimps in the control group. The results suggest the essential role of DE to enhance feed digestion and utilization, in order to optimize the feed effectiveness. Furthermore, this research also shows the potential to improve production efficiency by reducing the feed formulation costs ranging from 1.23 to 3.87% following the inclusion amount of PKM to partially replace SBM. This further emphasizes the importance of adding DE to support the production sustainability and profitability.

From the nutritional profile analysis of the whole body of the shrimp, this research demonstrated the functional role of DE to improve protein and fat retention compared to the control shrimp, and the group of shrimps fed with PKM without DE. For protein, better retention in the whole body of shrimp fed with PKM and DE can also be seen in the amino acid composition of the shrimp (table V). The study from Shamsuddin et al. (2021) shows that the more PKM is used in shrimp feed formulation, the more protein retention in the shrimp body will decrease. According to Akinyeye et al. (2011) and Thakur et al. (2019), the presence of anti-nutritional factors (ANFs) in PKM, such as phytic acid, tannins and oxalate, which can also cause a bitter taste, is responsible for the shrimp's low ability to absorb and utilize the nutrients from PKM. In addition, the high content of non-starch polysaccharides found in PKM will increase the intestinal viscosity, thereby reducing the absorption of nutrients (Ng, 2004). However, the outcomes of current research using DE show different results. The better retention of nutrient in the shrimp body could be due to the presence of beneficial microbiota activated by the inclusion of DE, that greatly influence the nutrient absorption process, and strengthen the shrimp's digestive health (Li et al., 2018), thus facilitating better absorption of nutrients from PKM. Furthermore, the availability of a monomeric form of threonine and lysine may allow better uptake of nutrients compared to the polymer form of proteins that can significantly impact the nutrient utilization and assimilation in the body of shrimp.

Currently, research on feed formulation in shrimp is also linked to the health condition of the shrimp, especially when substituting premium raw materials with economical raw materials (Novriadi, Hasan et al., 2023a, 2024a). This underscores the need for further research, particularly when replacing the premium ingredients with alternative ingredients contained with high levels of fibre, anti-nutritional factors, and the possibility of toxic elements (Cai et al., 2014; Zhang et al., 2023; Novriadi et al., 2024c). One parameter that can be observed is the number of total haemocytes counted (THC), which reflects the status of the immune response in shrimp (Ji et al., 2009). This parameter becomes increasingly important since the use of inappropriate raw materials can cause stress conditions in aquatic organisms (Zhang et al., 2023), and lowering the immune response against pathogens. In this study, the THC of the Pacific whiteleg shrimp, *P. vannamei* exhibited an increasing trend with higher levels of PKM inclusion. However, the differences observed between treatments were not statistically significant. This suggests that including PKM in the diet, even up to 9%, does not adversely affect the shrimp's immune system. The absence of significant changes in THC indicates that the inclusion of PKM does not induce stress or negatively trigger the immune response, implying that PKM is a well-tolerated and acceptable dietary component for shrimp at these levels. It is, however, worth to note that the THC level in shrimp fed with PKM alone is numerically higher than shrimp fed with PKM and supplemented with DE. This pattern suggests that DE supplementation may improve the digestibility of PKM, reducing potential negative impacts from the shrimp's inability to fully digest components of the PKM. The enhanced digestibility might reduce the immune stimulation associated with undigested feed, which could explain the lower THC levels in the DE-supplemented group. This indicates that while PKM is well-tolerated, its combination with DE could further optimize its use in shrimp diets by minimizing any subtle, stress-related immune responses.

Haemocytes in the circulating haemolymph in shrimp play a significant role in performing functions such as recognition, phagocytosis, coagulation, and encapsulation in the crustacean (Johansson et al., 2000; Liu et al., 2021). The number of haemocytes rises throughout the life of the shrimp in a special haemocyte-forming tissue called haematopoietic tissue (Lin & Söderhäll, 2011). However, whether fibre or polysaccharides contained in PKM or supplementation of DE can directly induce the formation and release of haemocytes in shrimp is not yet established, but may likely stimulate their release due to the presence of mannan-type complex oligosaccharide cell wall components. More than 81% of the total carbohydrates in palm kernel meal consist of non-starch polysaccharides mostly as β-(1,4)-D-mannans (Stein et al., 2015). Choct (2015) comprehensively reviewed the importance of appreciating the true fibre content of grains, pulses and other plant-based ingredients, which is the sum of non-starch polysaccharides and lignin, of feed. This is a fundamental prerequisite for animal nutritionists to improve the accuracy of feed formulation in the future. Thus, to further understand the functionality of DE in combination with PKM to improve the shrimp immune system, primarily THC, more comprehensive research, needs to be carried out by conducting challenge tests with specific pathogens or analysing the level of circulating THC at different points in time during the culture period.

This research also analysed lysozyme activity, as an important enzyme in the innate immune system of animals, including shrimp (Hikima et al., 2003), which plays a crucial role in defending the shrimp against bacterial infection by breaking down the cell walls of pathogens (Jollès & Jollès, 1984; Qasba et al., 1997). The evaluation results presented in fig. 2 show no significant statistical differences, although lysozyme activity values tended to become notably lowered in the shrimp-fed PKM without the DE addition, this may indicate less induction for lysozyme in the humoral defence pathways. With DE addition, however, there appeared to be a more pronounced lysozyme activity for PKM diets with DE inclusion in *P. vannamei*. Again, this modulatory effect could be attributed to the digestion enhancer degrading cell wall structures and releasing mannan type β-glycan oligosaccharide fragments.

Some studies showed that both oregano and lemongrass essential oils have bioactive components with antimicrobial, antioxidant, and immunomodulatory properties (Al-Sagheer et al., 2018; Valdivieso-Ugarte et al., 2019; Novriadi et al., 2023c). Oregano oils, which contain active compounds like carvacrol and thymol that have strong antibacterial properties, could stimulate the enhancement of lysozyme activity (Coelho et al., 2021; Novriadi et al., 2023c). Meanwhile, lemongrass essential oil contains compounds like citral that also exhibit antimicrobial activity and may help to induce the non-specific immune function in shrimp (Lam et al., 2023). Incorporating these essential oils into the DE could synergistically promote better immune function against bacterial infection and may also indirectly modulate the gut microbiome and promote improved hepatopancreas function and gastrointestinal integrity. Future research work could address this area of interest for such products.

CONCLUSION

This research shows that dietary inclusion of DE can constitute the suitable additive(s) to increase the proportion of plant protein in shrimp feeds, thereby reducing feed costs, and contrubite in mitigating the potential impacts of undigested dietary components on the health and immune system of the Pacific whiteleg shrimp, *Penaeus vannamei*. An inclusion level of DE as modest as 0.2% in feed formulated with graded inclusion levels of PKM, namely 2.5, 6 and 9% to partially reduce the inclusion of fish meal and soybean meal, is still capable of maintaining the optimal growth and health of the shrimp, while at the same time increasing the production efficiency by reducing feed formulation costs by 1.23-3.87%. In this way, the profitability and sustainability of shrimp production can continue to be increased.

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