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EFFECT OF AQUEOUS MORINGA (*MORINGA OLEIFERA*) LEAF EXTRACT AS A PREBIOTIC ON GROWTH OF THE WHITELEG SHRIMP, *PENAEUS VANNAMEI* BOONE, 1931 (DECAPODA, PENAEIDAE)

BY

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ABSTRACT

This study evaluated the potential of the aqueous moringa (*Moringa oleifera*) leaf extract (ME) as a prebiotic and its effect on the growth performance of the whiteleg shrimp, *Penaeus vannamei* Boone, 1931. The growth trial of probiotics in the broth containing ME showed that *Lactobacillus acidophilus* growth rate was significantly higher ($P<0.05$) than that of *Lactobacillus plantarum* and *Lactobacillus reuteri*. Furthermore, *L. acidophilus* exhibited also better growth ($P<0.05$) than *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* in the broth containing ME. The minimum inhibitory concentration of ME (MIC) and minimum bactericidal concentration (MBC) were the same against *V. alginolyticus*, *V. parahaemolyticus*, and *V. harveyi* at concentrations of 10, 7.5, and 7.5 mg/ml, respectively. By contrast, ME's MIC and MBC against *L. acidophilus* were 50 and >100 mg/ml, respectively. The prebiotic scores of *L. acidophilus* against *V. parahaemolyticus* and *V. harveyi* were significantly higher ($P<0.05$) than that against *V. alginolyticus*. The MIC, MBC, and prebiotic scores indicated that ME enhanced the growth of *L. acidophilus* more than that of *Vibrio* bacteria. Six diets were formulated for the feeding trial; 3 diets contained ME at 0 (ME0), 2.5 (ME2.5) and 5.0 g/kg (ME5.0), respectively, and the same 3 diets were also prepared with *L. acidophilus* (ME0 + P, ME2.5 + P, and ME5.0 + P, respectively). After the feeding trial, the number of lactic acid bacteria in the gut was higher ($P<0.05$) in the ME2.5 + P group than in the control and ME2.5 groups, but no differences were found in the vibrio-like bacterial count among the treatments. The ME2.5 + P group had a higher ($P<0.05$) final weight, weight gain, and specific growth rate than did the ME or *L. acidophilus* alone and the control groups. Therefore, ME exhibits a prebiotic function and exerts a symbiotic effect with *L. acidophilus*, thus increasing the growth performance of *P. vannamei*.

Key words. — *Moringa oleifera*, *Lactobacillus acidophilus*, antibacterial activity, prebiotic score, symbiotic effect, *Penaeus vannamei*

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RÉSUMÉ

Cette étude a évalué le potentiel de l'extrait aqueux de feuille (ME) de Moringa (*Moringa oleifera*) comme probiotique et ses effets sur les performances de croissance de la crevette à pattes blanches, *Penaeus vannamei* Boone, 1931. Les essais de croissance des probiotiques dans la solution contenant ME a montré que le taux de croissance de *Lactobacillus acidophilus* était significativement plus élevé ($P<0,05$) que celui de *Lactobacillus plantarum* et *L. reuteri*. De plus *L. acidophilus* a aussi montré une meilleure croissance ($P<0,05$) que *Vibrio alginolyticus*, *V. parahaemolyticus*, et *V. harveyi* dans la solution contenant ME. La concentration minimum inhibitrice de ME (MIC) et la concentration bactéricide minimale (MBC) ont été les mêmes contre *V. alginolyticus*, *V. parahaemolyticus*, et *V. harveyi* à des concentrations respectives de 10, 7,5, et 7,5 mg/mL. Par contre, ME's MIC et MBC contre *L. acidophilus* ont été respectivement de 50 et >100 mg/mL. Les scores probiotiques de *L. acidophilus* contre *V. parahaemolyticus* et *V. harveyi* ont été significativement plus élevés ($P<0,05$) que contre *V. alginolyticus*. MIC, MBC et les scores probiotiques ont montré que ME augmente la croissance de *L. acidophilus* plus que celle de la bactérie *Vibrio*. Six régimes ont été formulés pour l'essai d'alimentation ; 3 régimes contenant respectivement ME à 0 (ME0), 2,5 (ME2,5), et 5,0 g/kg (ME5,0), et les mêmes 3 régimes dans les groupes préparés respectivement avec *L. acidophilus* (ME0 + P, ME2,5 + P, et ME5,0 + P). Après l'essai d'alimentation, le nombre de bactéries lactiques dans le tube digestif a été plus élevé ($P<0,05$) dans le groupe ME2,5 + P que chez les témoins et le groupe ME2,5, mais aucune différence n'a été trouvée dans le nombre de bactéries type vibrion parmi les traitements. Le groupe ME2,5 + P a eu un poids final, un gain de poids et une croissance spécifique plus élevés ($P<0,05$) que celui des groupes ME, ou *L. acidophilus* seul, et les témoins. Donc, ME montre une fonction probiotique et exerce un effet symbiotique avec *L. acidophilus*, accroissant ainsi les performances de croissance de *P. vannamei*.

Mots clés. — *Moringa oleifera*, *Lactobacillus acidophilus*, activité antibactérienne, score probiotique, effet symbiotique, *Penaeus vannamei*

INTRODUCTION

The whiteleg shrimp, *Penaeus vannamei* Boone, 1931 (by some authors still referred to as *Litopenaeus vannamei* (Boone, 1931)), constitutes a commercially valuable shrimp and is, next to being caught in the wild, also raised in aquaculture for human consumption (Figueredo et al., 2023). As a consequence, aquaculturists, in particular in Southeast Asia, are constantly trying to improve culturing conditions of this species, in attempts to raise production and thus to enhance yields (Manan & Ikhwanuddin, 2021). One aspect of such improvements could be found in influencing the gut microbiota of *P. vannamei*, which may lead to better health conditions of the shrimp and thus enhance survival (El-Saadony et al., 2022). In the present study, the use of moringa for improving the gut microbiota of these shrimps has been investigated.

Moringa, *Moringa oleifera* Lamarck (Brassicales, Moringaceae), has many local names (including mother milk, drumstick tree, miracle tree) but its most common English name is horseradish tree. Moringa is originally from India and grown in subtropical and tropical regions worldwide (Foidl et al., 2002). Moringa grows rapidly, as much as 5 m/year (Morton, 1991), and is well known for its

nutritional content, including 27.12% crude protein, 2.33% crude lipid, 19.17% fibre, 6.1% ash, and 36.88% nitrogen-free extract, vitamins and minerals (Sherif et al., 2014). Thus, different parts of moringa, including the pods, leaves, seeds, gums, barks and flowers, are widely consumed as food or used as traditional medicine (Mahmood et al., 2010).

The water extract of moringa leaves has antibacterial properties that inhibit the growth of some bacteria, including *Escherichia coli* (Migula, 1895) Castellani & Chalmers, 1919, *Staphylococcus aureus* Rosenbach, 1984, and *Streptococcus pneumoniae* (Klein, 1884) Chester, 1901 (cf. Idris & Abubakar, 2016), but not that of other bacteria, such as *Bacillus pumilus* Meyer & Gottheil, 1901, *Bacillus cereus* Frankland & Frankland, 1887, and *Streptococcus faecalis* Andrewes & Horder, 1906 (cf. Moyo et al., 2012). The actual antibacterial properties of moringa depend on the solvent used during the extraction process: the antibacterial efficacy of the acetone and methanol extracts of moringa leaves is higher than that of aqueous extracts (Moyo et al., 2012; Idris & Abubakar, 2016). However, the aqueous extract offers numerous advantages as a green extraction solvent, because water is inexpensive, nontoxic, environmentally friendly, and provides an opportunity for clean processing and preventing pollution (Filly et al., 2016).

Moringa might act as a prebiotic in diets (Gbadebo et al., 2019; Li et al., 2021; Prayitno et al., 2021). Prebiotics are non-digestible nutrients that stimulate the increase of beneficial gut microbiota in the host, whereas probiotics are live microbial feed additives that beneficially affect the host; therefore, the prebiotic supports probiotic growth (Akhter et al., 2015). Ordaz et al. (2018) found that phenolic compounds in the plants selectively promote probiotic growth and inhibit pathogenic bacterial growth. Furthermore, Elabd et al. (2018) reported that polyphenols in herbs can modify the gut microbial composition.

Depending on the extraction technique, the number of phenolic compounds in the moringa leaf extract may vary from 2.3 to 13.5% (Vongsak et al., 2013). Moreover, the polysaccharide extract of the moringa leaf modulates microbial composition in the intestines (Li et al., 2021). A study reported a decrease in the numbers of *E. coli*, *Salmonella* and *Staphylococcus* spp. but an increase in the number of *Lactobacillus* in the small intestine of broiler chickens, *Gallus gallus* (Linnaeus, 1758), fed with moringa (Hafsa et al., 2019). Moreover, moringa supplementation in the diet of mice (*Mus musculus* Linnaeus, 1758) affects the gut microbiota composition, increases the number of *Lactobacillus*, and reduces the number of *Bifidobacteria* (Elabd et al., 2018). Wang et al. (2019) demonstrated that the polysaccharide extract of the moringa leaf exerts a prebiotic effect by maintaining the integrity of the intestinal mucosa and modulating the gut microbiota composition. Therefore, moringa may function as a prebiotic in the whiteleg shrimp, *Penaeus vannamei*.

A plant is classified as a prebiotic if it selectively stimulates the growth of beneficial bacteria; consequently, intestinal microorganisms can ferment the prebiotic, and the prebiotic resists host digestion (Gibson et al., 2017). Thus, inclusion of moringa in the diet of whiteleg shrimp can promote the growth of beneficial bacteria, such as *Lactobacillus*, and reduce the growth of pathogenic bacteria, such as *Vibrio*. Prebiotic activity can be determined in vitro by comparing the growth of beneficial bacteria with that of enteric pathogens in the presence and absence of the given prebiotic in the medium (Mallik & Bhawsar, 2018).

Prebiotics affect not only the growth of beneficial bacteria but also the health and growth performance of animals (Ringo et al., 2010). For example, the addition of prebiotic honey in *Penaeus vannamei* diet increased their total weight and reduced the feed conversion ratio (Fuandila et al., 2020). Furthermore, supplementation of prebiotics and probiotics in the diet of *P. vannamei* resulted in improved growth compared with the use of a prebiotic or probiotic alone (Arisa et al., 2015).

To the best of our knowledge, no study has evaluated the antimicrobial and prebiotic activity of the aqueous moringa leaf extract (ME) against shrimp pathogenic bacteria. Therefore, this study evaluated the potential of the aqueous ME as a prebiotic by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), prebiotic score, as well as lactic acid bacterial count and vibrio-like count in the intestine. Additionally, this study investigated the effect of diet supplementation with the aqueous ME and *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 on the growth of *Penaeus vannamei*.

MATERIAL AND METHODS

ME preparation

Moringa oleifera leaves were purchased from Rumah Kelor, Blora, Indonesia. The leaves were prepared as previously described by Abidin et al. (2022b). The leaves were cleaned and air-dried for 3 days at 35°C. The dried leaves were grounded into a fine powder, mixed with distilled water (98°C) at a ratio of 1 : 9, and stored for 24 h at room temperature. The solution was filtered using a muslin cloth and Whatman Filter Paper No. 1 (Whatman, Brentford, U.K.) to separate the leaves from the liquid solution. The solution was then frozen at −80°C and dried in a freeze dryer for 3 days until the solution turned to powder. The powder was stored at −20°C until further use.

Bacterial preparation

Three probiotics (*Lactobacillus acidophilus*, *Lactobacillus reuteri* Kandler et al., 1982, and *Lactobacillus plantarum* (Orla-Jensen, 1919) Bergey et al., 1923)

and 3 *Vibrio* bacteria (*Vibrio alginolyticus* (Miyamoto et al., 1961) Sakazaki, 1968, *Vibrio harveyi* (Johnson & Shunk, 1936) Baumann et al., 1981, and *Vibrio parahaemolyticus* (Fujino et al., 1951) Sakazaki et al., 1963) were used in the present study. These bacteria were provided by Professor Liu Ping-Chung's laboratory (Department of Aquaculture, National Taiwan Ocean University, Taiwan).

Three probiotic stocks were individually grown on De Man, Rogosa, and Sharper Agar (MRS, Neogen, Lansing, MI, U.S.A.) containing 2% NaCl at 28°C for 24 h. One colony was scaled up in MRS broth containing 2% NaCl at 28°C for 24 h. Moreover, the 3 *Vibrio* bacteria were individually grown on thiosulfate-citrate-bile salt-sucrose (TCBS) agar (Neogen) that was supplemented with 2% NaCl at 28°C for 24 h. One colony was selected and transferred to tryptic soy broth (Neogen) that was supplemented with 2% NaCl. Each of the bacterial solutions was adjusted to 1×10^9 CFU/ml for further use.

Bacterial growth in ME

To prepare the bacterial broths, we transferred 10 μl of each probiotic stock to 10 ml of MRS broth supplemented with the ME concentrations of 0, 0.25, 0.50, and 1.00 mg/ml. The probiotics were then incubated at 28°C for 24 h. The probiotic with the highest growth rate at low ME concentrations was selected for the next step of the experiment. The selected probiotic, namely *Lactobacillus acidophilus*, was then cultured in different ME concentrations (0, 5, 10, 25, 50, 75, and 100 mg/ml) to determine the optimal ME concentration for bacterial growth. The concentration that resulted in the superior growth of *L. acidophilus*, namely 5 mg/l, was used in the growth test of *Vibrio* bacteria.

A total of 10 μl stock of each *Vibrio* and *L. acidophilus* was transferred to 10 ml of broth containing 5 mg/ml of the ME. The bacteria were incubated at 28°C for 48 h. Bacterial growth was measured at 6, 12, 24, and 48 h.

To count the bacteria, a bacterial solution of *L. acidophilus* (200 μl) and 200 μl of solutions of each *Vibrio* were spread on MRS and TSA agar, respectively, then and incubated for 24 h at 28°C. All the experiments were conducted in triplicate.

MIC and MBC tests

The MIC and MBC were determined using the broth dilution method previously described by Unegbu et al. (2020). The ME was dissolved in broth to obtain the ME concentrations of 0, 5, 7.5, 10, 25, 50, 75 and 100 mg/ml. Stock of each bacterium (*L. acidophilus*, *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi*; 10 μl each) was transferred into a 10-ml broth containing various ME concentrations. One of each ME concentration was not inoculated with bacteria and served as a negative

control. All bacteria were incubated at 28°C for 24 h. The lowest ME concentration that indicated no visual turbidity change was considered as the MIC.

After the MIC was determined, 200 μl of bacteria from all the tubes with no visible bacterial growth were seeded on the MRS (for *L. acidophilus*) and TSA (for *Vibrio* bacteria) and incubated at 28°C for 24 h. The lowest ME concentration that exhibited no bacterial colony on the agar was considered as the MBC. In each case, three experiments were conducted in parallel.

Prebiotic activity score

The prebiotic activity score was determined using the method previously reported by (Thitiratsakul & Anprung, 2014) with some modifications. *L. acidophilus* was the representative probiotic, and *Vibrio* bacteria were the pathogenic species. A 10-μl bacterial volume was taken from the stock and inoculated in 10 ml of broth containing 5 mg/ml of ME or glucose for 24 h at 28°C. All the bacterial colonies grown on TSA at 28°C were counted. The prebiotic activity score was determined using the following equation:

$$\text{Prebiotic score} = \frac{\log \mathrm{P}_{\mathrm{m}}^{24} - \log \mathrm{P}_{\mathrm{m}}^{0}}{\log \mathrm{P}_{\mathrm{g}}^{24} - \log \mathrm{P}_{\mathrm{g}}^{0}} - \frac{\log \mathrm{E}_{\mathrm{m}}^{24} - \log \mathrm{E}_{\mathrm{m}}^{0}}{\log \mathrm{E}_{\mathrm{g}}^{24} - \log \mathrm{E}_{\mathrm{g}}^{0}}$$

where P = *L. acidophilus*, m = in *M. oleifera*, 0 = initial CFU ml/ml, E = *Vibrio* bacteria, g = in glucose and 24 = final CFU ml/ml.

Dietary ME preparation

Six experimental diets were formulated. The diets contained either both ME and *L. acidophilus* or only ME or *L. acidophilus* alone (table I). Three diets contained ME at 0, 2.5, and 5.0 g/kg, which were denoted as ME0, ME2.5, and ME5.0, respectively. The same 3 diets were sprayed with *L. acidophilus* at a concentration of 1×10^7 CFU/g diet, which were denoted as ME0 + P, ME2.5 + P, and ME5.0 + P, respectively. These diets were dried for 24 h at 35°C and stored at 4°C until further use. The proximate compositions of the diet (i.e., lipid, crude protein, ash, and moisture) were analysed using standard methods (Helrich, 1990).

Lactic acid and *Vibrio*-like bacterial count in the gut

Eighteen healthy shrimp, *Penaeus vannamei* (14.5 $\pm$ 0.3 g) were randomly distributed into six 75-litre tanks. The shrimp were fed with the experimental diets for 7 days. On the final day, the shrimp were starved for 12 h and dissected to retrieve and weigh the whole gut, which included the foregut (stomach), midgut and hindgut. The whole gut was homogenized in phosphate-buffered saline at a ratio of 1 : 9 (w/v). To count lactic acid and *Vibrio*-like bacteria, 200 μl of the

TABLE I
Ingredients of the experimental diets used in this study

Ingredient (g/kg diet)	ME0	ME0 + P	ME2.5	ME2.5 + P	ME5.0	ME5.0 + P
Fish meal	500	500	500	500	500	500
Shrimp meal	60	60	60	60	60	60
Yeast	50	50	50	50	50	50
ME	0	0	2.5	2.5	5	5
L. acidophilus (CFU/g)	–	1×10^7	–	1×10^7	–	1×10^7
α-starch	150	150	150	150	150	150
Fish oil	15	15	15	15	15	15
Lecithin	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5
Choline chloride	5	5	5	5	5	5
Vitamin D_3	1	1	1	1	1	1
Vitamin E	1	1	1	1	1	1
Vitamin A	1	1	1	1	1	1
Vitamin premix[a]	40	40	40	40	40	40
Mineral premix[b]	40	40	40	40	40	40
α-cellulose	127	127	124.5	124.5	122	122
Proximate analysis (% in dry weight)						
Crude protein	50.11		50.65		50.84	
Crude lipid	8.79		8.78		8.74	
Ash	11.45		11.57		11.74	
Moisture	7.8		7.5		7.2	

[a]Vitamin premix includes 0.5% thiamin HCl, 0.8% riboflavin, 2.6% niacinamide, 0.1% D-biotin, 1.5% Ca-pantothenate, 0.3% pyridoxin HCl, 0.5% folic acid, 18.1% inositol, 3% para-aminobenzoic acid, 0.1% cyanocobalamin, 0.1% BHT, 60.3% α-cellulose and 12.1% ascorbic acid.
[b]Mineral premix includes 2.1% calcium carbonate, 73.5% calcium phosphate dibasic, 0.227% citric acid, 0.046% cupric acid, 0.558% ferric acid (16-17% Fe), 2.5% magnesium oxide, 0.825% magnesium citrate, 6.8% potassium iodide sulphate, 3.06% sodium chloride, 2.14% sodium phosphate, 0.133% zinc citrate, 0.001% potassium iodine and 8. 1% potassium phosphate dibasic.

homogenized gut was spread on MRS and TCBS agar, respectively, and incubated at 28°C for 48 and 24 h, respectively. The colonies that appeared on the agar were evaluated.

Growth performance

In total, 180 juvenile whiteleg shrimp (2.00 ± 0.25 g) were randomly distributed into eighteen 75-litre tanks (6 groups with 3 replications per group). The shrimp were reared in a recirculation system for 30 days and fed 3 times a day with the experimental diet at 5% body weight. The shrimp were weighed on day 14, and the amount of feed was adjusted accordingly. Water was maintained at the following conditions: a temperature of 28-29°C, oxygen >6 mg/l, salinity of 30 to 33‰, and pH of 6.8 to 7.6. The growth parameters were measured on the final day by using

the following equations:

$$\text{Weight gain}(\%) = \big((\text{final weight (g)} - \text{initial weight (g)})/\text{initial weight (g)}\big) \times 100$$

$$\text{Feed conversion rate (FCR)} = (\text{consumed diet (g)}/\text{biomass gain (g)} \times 100$$

$$\text{Specific growth rate (SGR; \% per day)} = \big((\ln(\text{final weight}) \text{ (g)} - \ln(\text{initial weight}) \text{ (g)})/\text{time (days)}\big) \times 100$$

$$\text{Survival rate (\%)} = \big((\text{number of individuals at the end of the trial}/\text{initial number of individuals stocked})\big) \times 100$$

Statistical analysis

The experiments were arranged in a randomized set. Shapiro-Wilk and Levene's tests were used to test the normality and homogeneity of the data before data were subjected to one-way analysis of variance (ANOVA). Duncan's test was used to determine significant differences among the treatments. A P value of <0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Bacterial growth in ME

Lactobacillus plantarum, *Lactobacillus reuteri* and *Lactobacillus acidophilus* were grown in ME at 28°C for 24 h. The growth of *L. acidophilus* was significantly higher ($P<0.05$) than that of the other bacteria in the aqueous ME at a concentration of 0.25 mg/ml. Additionally, at the ME concentrations of 0. 50 and 1.00 mg/l, *L. plantarum* and *L. acidophilus* exhibited significantly better growth ($P<0.05$) than did *L. reuteri* ($P<0.05$; fig. 1). Therefore, *L. acidophilus* was used as the probiotic in the present study because it demonstrated the highest growth performance even at a low ME concentration.

An ME concentration of 5 to 25 mg/l resulted in better growth ($P<0.05$) than did ME concentrations of 0 and of 75 to 100 mg/l (fig. 2). The growth of *L. acidophilus* tended to decrease at ME concentrations above 25 mg/l.

Fig. 3 shows that the growth of *L. acidophilus* was significantly lower ($P<0.05$) than that of the other three *Vibrio* bacteria at 6 and 12 h ($P<0.05$). However, at 24 h, the growth of *L. acidophilus* was higher ($P<0.05$) than that of both *Vibrio harveyi* and *Vibrio parahaemolyticus* but lower than that of *Vibrio alginolyticus* ($P<0.05$). At 48 h, the ME resulted in higher growth ($P<0.05$) of *L. acidophilus* compared with the three *Vibrio* bacteria.

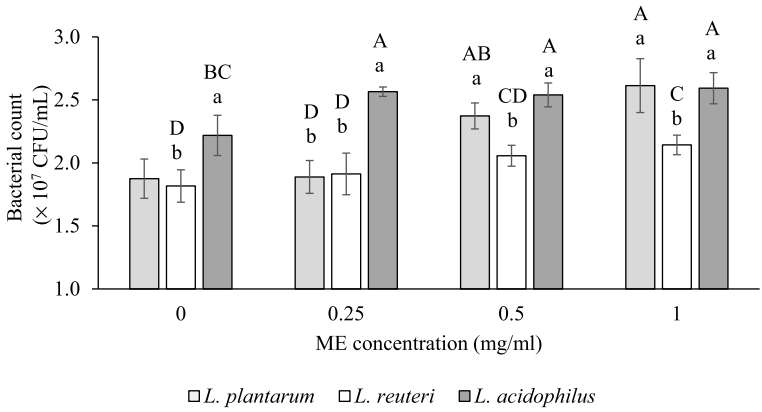

Fig. 1. Growth of probiotics cultured in ME concentrations of 0, 0.25, 0. 50 and 1.00 mg/ml at 28°C for 24 h. Data are expressed as the mean ± standard deviation ($n = 3$). Bars with different lowercase letters (a, b) are significantly different ($P<0.05$) at the same concentration. Bars with different uppercase letters (A, B, C, D) are significantly different ($P<0.05$) at all concentrations. The bacteria at issue are: *Lactobacillus plantarum* (Orla-Jensen, 1919) Bergey et al., 1923), *Lactobacillus reuteri* Kandler et al., 1982, and *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970.

MIC and MBC

As shown in table II, the MIC of ME for *L. acidophilus* was 50 mg/ml, and the bacterium remained alive even at an ME concentration of 100 mg/ml. However, the MIC and MBC values were the same at the ME concentrations of 10.0, 7.5 and 7.5 mg/l for *V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi*, respectively.

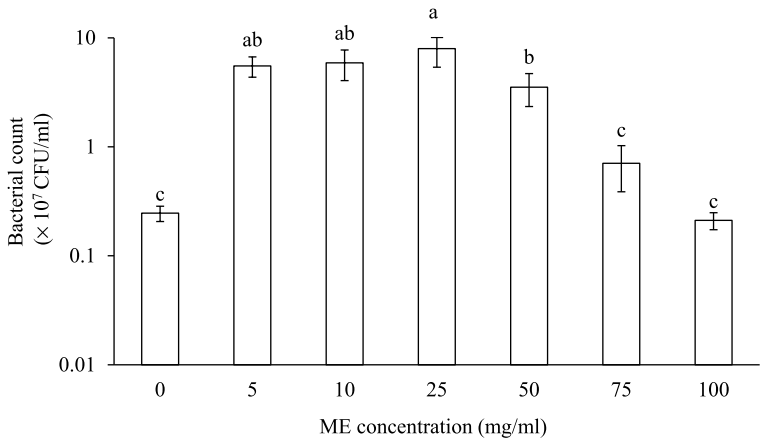

Fig. 2. Growth of *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 cultured in ME concentrations of 0, 5, 10, 25, 50, 75 and 100 mg/ml at 28°C for 24 h. Data are expressed as the mean ± standard deviation ($n = 3$). Bars with different letters (a, b, c) are significantly different ($P<0.05$).

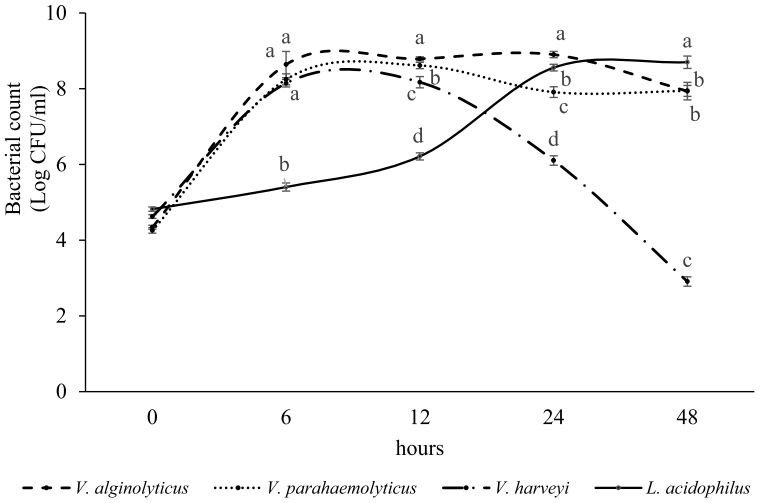

Fig. 3. Number of *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 and *Vibrio* spp. cultured in an ME concentration of 5 mg/ml at 28°C for 48 h. Data are expressed as the mean ± standard deviation ($n = 3$). Dots with different letters (a, b, c, d) at the same hour are significantly different ($P < 0.05$). The *Vibrio* species under concern are: *Vibrio alginolyticus* (Miyamoto et al., 1961) Sakazaki, 1968, *Vibrio parahaemolyticus* (Fujino et al., 1951) Sakazaki et al., 1963), and *Vibrio harveyi* (Johnson & Shunk, 1936) Baumann et al., 1981.

Therefore, ME inhibited the growth of *Vibrio* more effectively than that of *L. acidophilus*.

Prebiotic activity score

The prebiotic scores were positive for all the bacteria (table III). The prebiotic activity of ME was significantly higher in *V. parahaemolyticus* and *V. harveyi* than

TABLE II

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ME against *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 and three *Vibrio* bacteria, *Vibrio alginolyticus* (Miyamoto et al., 1961) Sakazaki, 1968, *Vibrio parahaemolyticus* (Fujino et al., 1951) Sakazaki et al., 1963 and *Vibrio harveyi* (Johnson & Shunk, 1936) Baumann et al., 1981

	MIC (mg/ml)	MBC (mg/ml)
L. acidophilus	50	>100*
V. alginolyticus	10	10
V. parahaemolyticus	7.5	7.5
V. harveyi	7.5	7.5

**L. acidophilus* colonies were still observed on MRS at a ME concentration of 100 mg/ml.

TABLE III

ME prebiotic activity score: *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 compared against three *Vibrio* bacteria, *Vibrio alginolyticus* (Miyamoto et al., 1961) Sakazaki, 1968, *Vibrio parahaemolyticus* (Fujino et al., 1951) Sakazaki et al., 1963 and *Vibrio harveyi* (Johnson & Shunk, 1936) Baumann et al., 1981

	Prebiotic score
L. acidophilus against *V. alginolyticus*	0.85 ± 0.14^{b}
L. acidophilus against *V. parahaemolyticus*	2.25 ± 0.37^{a}
L. acidophilus against *V. harveyi*	2.09 ± 0.26^{a}

Data are expressed as the mean ± standard deviation ($n = 3$). Means in the same column with different letters (a, b) are significantly different ($P < 0.05$).

in *V. alginolyticus* ($P < 0.05$). Thus, ME promoted the growth of *L. acidophilus* but suppressed the growth of *V. harveyi*, *V. parahaemolyticus* and *V. alginolyticus*.

Lactic acid and *Vibrio*-like bacterial counts in gut

After feeding for seven days, the ME2.5 + P group had a higher ($P < 0.05$) lactic acid bacterial count than did the ME2.5 group; however, no difference was observed between the ME2.5 + P and ME5.0 + P groups. The total lactic acid bacterial count did not differ between the shrimp fed with the ME-supplemented diet and the shrimp fed with the control diet. No differences were noted in the total lactic acid bacterial count between the ME5.0 + P group and the other groups, except for the control group (fig. 4a). Moreover, supplementation of ME and *L.*

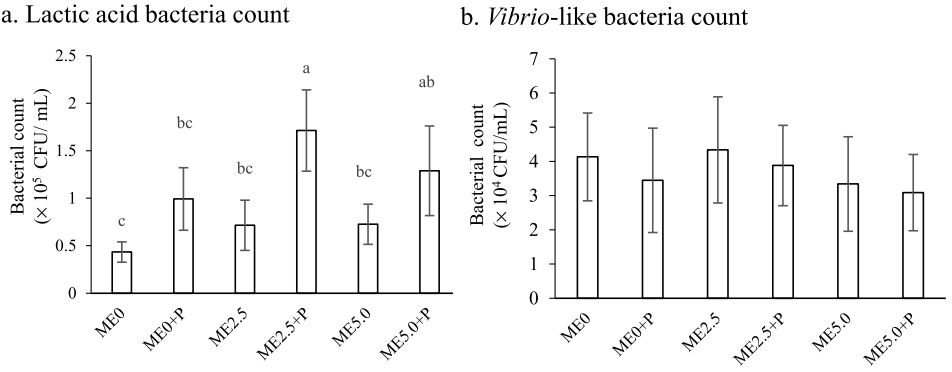

Fig. 4. Number of total: a, lactic acid bacteria; and b, *Vibrio*-like bacteria in the gut of whiteleg shrimp, *Penaeus vannamei* Boone, 1931, fed diets containing ME and *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 individually or combined. Data are expressed as the mean ± standard deviation ($n = 3$). Bars with different letters (a, b, c) are significantly different ($P < 0.05$).

TABLE IV

Growth performance of *Penaeus vannamei* Boone, 1931 fed diets supplemented with ME and *Lactobacillus acidophilus* (Moro, 1900) Hansen & Mocquot, 1970 for 30 days

Diet	Initial weight (g)	Final weight (g)	Weight gain (%)	SGR (%/day)	FCR	SR (%)
ME0	2.04 ± 0.02^{a}	4.6 ± 0.10^{d}	123 ± 5.9^{d}	2.6 ± 0.09^{d}	1.50 ± 0.10^{b}	100 ± 0.0^{a}
ME0 + P	2.04 ± 0.03^{a}	4.8 ± 0.11^{c}	136 ± 0.3^{c}	2.8 ± 0.00^{c}	1.36 ± 0.11^{a}	100 ± 0.0^{a}
ME2.5	2.07 ± 0.04^{a}	5.4 ± 0.15^{b}	165 ± 3.7^{b}	3.2 ± 0.04^{b}	1.43 ± 0.05^{a}	100 ± 0.0^{a}
ME2.5 + P	2.05 ± 0.02^{a}	5.8 ± 0.15^{a}	187 ± 10.4^{a}	3.5 ± 0.11^{a}	1.36 ± 0.05^{a}	100 ± 0.0^{a}
ME5.0	2.01 ± 0.01^{a}	5.4 ± 0.05^{b}	170 ± 4.7^{b}	3.3 ± 0.05^{b}	1.30 ± 0.10^{a}	100 ± 0.0^{a}
ME5.0 + P	2.03 ± 0.04^{a}	5.6 ± 0.11^{b}	178 ± 12.6^{ab}	3.4 ± 0.15^{ab}	1.33 ± 0.05^{a}	100 ± 0.0^{a}

See text for further explanation of the various diets. Data are presented as the mean ± standard deviation ($n = 3$). Means in the same column with different letters (a, b, c, d) are significantly different ($P < 0.05$). SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate.

acidophilus, either individually or in a combined diet, did not affect the total number of *Vibrio*-like bacteria in the gut of *Penaeus vannamei* (fig. 4b).

Growth performance

The final weight, weight gain, and SGR were significantly higher ($P < 0.05$) in the ME2.5 + P group than in the other groups (table IV). No differences in growth performance parameters were observed between the ME5.0 and ME5.0 + P groups. The ME0 + P group exhibited a higher final weight, weight gain, and SGR than did the ME0 group. Furthermore, weight gain and SGR did not significantly differ between the ME2.5 + P and ME5.0 + P groups. No significant difference in the FCR was noted among all the treatment groups; however, all treatment groups had considerably higher FCRs than did the control group ($P < 0.05$).

DISCUSSION

Prebiotics are mainly derived from disaccharides and simple polysaccharides (Yoo et al., 2012). For instance, polysaccharides from mushrooms promote the growth of *Lactobacillus* (Nowak et al., 2018). Prebiotics are commonly associated with nondigestible carbohydrate fibres; however, in the last 10 years, the polyphenolic compound has garnered attention as a potential prebiotic (Chen et al., 2015). Furthermore, Milutinović et al. (2021) reported that polyphenols are poorly digestible and considered a novel prebiotic group. The aqueous ME contains approximately 6.84% polysaccharides (Li et al., 2020) and has a high total phenolic content of 2.35 to 4.41%.

The present study revealed that *Lactobacillus acidophilus* and *Lactobacillus plantarum* demonstrated higher growth performance among the three *Lactobacillus* tested. One study revealed that various *Lactobacillus* species exhibit different growth responses even if they are cultured in the same herb extract (Milutinović et al., 2021). Moreover, different herb extracts yield different growth rates of *Lactobacillus* (Nanasombat et al., 2018). The growth performance of *L. acidophilus* is higher than that of *Streptococcus thermophilus* Orla-Jensen, 1919 and *Bifidobacterium animalis* (Mitsuoka, 1969) Scardovi & Trovatelli, 1974 emend. Masco et al., 2004 in banana (*Musa acuminata* Colla) peel fibre powder (Santo et al., 2012). Additionally, mangosteen extract (*Garcinia mangostana* Linnaeus, 1753) resulted in a higher growth performance of *L. acidophilus* than did gac extract (*Momordica cochinchinensis* (Lour.) Spreng.) (Nanasombat et al., 2018). The results of the present study indicate that ME as an aqueous solution has prebiotic potential and is suitable for enhancing the growth of *L. acidophilus*.

An ME concentration of 5 to 25 mg/ml yielded the maximum growth of *L. acidophilus*. A mangosteen, nutgrass (*Cyperus rotundus* Linnaeus, 1753), and gac extract concentration of 5 mg/l increased the growth of *L. acidophilus* (Nanasombat et al., 2018). The ME extract did not inhibit the growth of *Vibrio parahaemolyticus* and *V. alginolyticus* after 24 h of incubation. However, the growth of the two *Vibrio* bacteria decreased at 48 h, and the growth of *L. acidophilus* increased. Compared with the growth of other *Vibrio* bacteria, the growth of *V. harveyi* was more strongly inhibited by ME.

We determined the same value of the MIC and MBC for *V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi* at the ME concentrations of 10.0, 7.5 and 7.5 mg/ml, respectively. Another study revealed that the MIC and MBC of the aqueous extract of moringa leaf for *Staphylococcus aureus* were both 25 mg/ml, and the MIC and MBC for *Escherichia coli* were 12.5 and 50 mg/ml, respectively (Unegbu et al., 2020). The MIC and MBC are used to determine the presence of antibacterial activity; therefore, the current results revealed that the aqueous ME exhibits antibacterial activity against *Vibrio*. Mogana et al. (2020) demonstrated that an MBC:MIC ratio of ≤ 4 is bactericidal and an MBC:MIC ratio of >4 is bacteriostatic. Because the MIC and MBC had the same values, the ME is considered bactericidal for the *Vibrio* bacteria. The antimicrobial activity of the aqueous ME can be attributed to the presence of secondary metabolites, including phenols, flavonoids, steroids, glycosides, tannins, saponins, alkaloids, anthraquinones, and terpenoids (Unegbu et al., 2020). However, high MIC and MBC values for *L. acidophilus* indicate that high ME concentrations are bacteriostatic and not effective against *L. acidophilus*.

A low or negative prebiotic score means that the growth of the tested strain is lower on a specific prebiotic than on glucose or its growth is lower than that

of the reference enteric bacteria (Huebner et al., 2007). A lower ME prebiotic score for *V. alginolyticus* compared with for *V. harveyi* and *V. parahaemolyticus* revealed that the ME inhibits the growth of *V. harveyi* and *V. parahaemolyticus* more than that of *V. alginolyticus*. However, a positive prebiotic score demonstrated that *L. acidophilus* grows more after metabolizing the ME than after metabolizing glucose. Moreover, *L. acidophilus* grows more effectively than the three *Vibrio* bacteria that metabolized the ME.

Administering probiotics and prebiotics in *Penaeus vannamei* affects the composition of the gut microbiota (Munaeni et al., 2020; Patil et al., 2021). Diet supplementation with ME and *L. acidophilus* in the ME2.5 + P and ME5.0 + P groups increased the presumptive lactic acid bacterial counts. The higher lactic acid bacterial count in the ME2.5 + P group indicated that the ME extract supports the growth of lactic acid bacteria in the gut. Prabawati et al. (2022) revealed that the symbiotic relationship between oyster mushrooms (*Pleurotus ostreatus* (Jacq.) P. Kumm., 1871) and *L. plantarum* significantly increased the number of lactic acid bacteria in the intestine; this finding is similar to that of our study.

The number of *Vibrio*-like bacteria did not significantly differ among the groups after feeding for 7 days. Imaizumi et al. (2021) reported that the probiotic diet did not alter the *Vibrio* bacterial count in the stomach and midgut after *Penaeus vannamei* were fed for 1 and 2 weeks. However, a study demonstrated that after 60 days of feeding, the *Vibrio* bacterial abundance was lower among shrimp fed with a diet supplemented with ME and *L. acidophilus* than among shrimp fed with a diet supplemented with ME alone (Abidin et al., 2022a). Another study indicated that the symbiotic effect of β-glucan with *Bacillus subtilis* (Ehrenberg, 1835) Cohn, 1872 reduced the number of *Vibrio* spp. and increased the number of lactic acid bacteria in whiteleg shrimp after 90 days of feeding (Boonanuntanasarn et al., 2016). However, the presence of *Vibrio* bacteria does not always affect shrimp, because some *Vibrio* bacteria are endogenous in the *P. vannamei* intestine (Zoqratt et al., 2018).

Incorporating moringa in diets increases the growth performance of some aquatic animals, such as the whiteleg shrimp, *Penaeus vannamei* (cf. Akbary et al., 2021; Abidin et al., 2022b), the giant freshwater prawn (*Macrobranchium rosenbergii* (De Man, 1879)) (cf. Kaleo et al., 2019), and nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) (cf. Richter et al., 2003; Astuti et al., 2007; Hamed & Sayed, 2019; Gawad et al., 2020). Moreover, also inclusion of *L. acidophilus* in diets increased the growth performance of *P. vannamei* (cf. Wang & Gu, 2010; Liu et al., 2016; Zeng, 2019). The current study revealed that diet supplementation with ME and *L. acidophilus*, either individually or combined, increased the growth performance of *P. vannamei*. The ME2.5 + P group demonstrated higher growth performance than did the ME2.5 group, which indicated a symbiotic relationship

between ME and *L. acidophilus*. The interaction of herbs and probiotics occurs when bacteria digests herbs into small molecules, which can then be easily absorbed by the host cell (An et al., 2019). Furthermore, the symbiotic effect between prebiotics and probiotics increases the villi height of the intestine of *P. vannamei* and the digestive enzyme activity (Yu et al., 2009; Boonanuntanasarn et al., 2016). Consequently, nutrient absorption and growth performance were improved in shrimp that were fed a diet containing ME and *L. acidophilus*.

In conclusion, the aqueous ME stimulates the growth of *L. acidophilus* and demonstrates antibacterial activity against *Vibrio alginolyticus*, *V. parahaemolyticus*, and *V. harveyi*. The ME demonstrates prebiotic activity and exerts a symbiotic effect that increases the growth performance of *Penaeus vannamei* when the ME is supplemented in diets at a concentration of 2.5 g/kg.

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REFERENCES

ABIDIN, Z., H. T. HUANG, Y. F. HU, J. J. CHANG, C. Y. HUANG, Y. S. WU & F. H. NAN, 2022a. Effect of dietary supplementation with *Moringa oleifera* leaf extract and *Lactobacillus acidophilus* on growth performance, intestinal microbiota, immune response, and disease resistance in whiteleg shrimp (*Penaeus vannamei*). Fish Shellfish Immunol., **127**: 876-890.

ABIDIN, Z., H. T. HUANG, Z. H. LIAO, B. Y. CHEN, Y. S. WU, Y. J. LIN & F. H. NAN, 2022b. *Moringa oleifera* leaves extract enhances nonspecific immune responses, resistance against *Vibrio alginolyticus*, and growth in whiteleg shrimp (*Penaeus vannamei*). Animals, **12**: 42.

AKBARY, P., A. GHOLAMHOSSEINI, M. ALI, A. JAHANBAKHSHI, K. R. TAVABE, B. S. KUCHAKSARAEI & A. T. MIRGHAED, 2021. Dietary administration of *Moringa oleifera* extract enhances growth, fatty acid composition, antioxidant activity and resistance of shrimp *Litopenaeus vannamei* against *Photobacterium damselae*. Agricult. Nat. Resourc., **55**: 161-170.

AKHTER, N., B. WU, A. M. MEMON & M. MOHSIN, 2015. Probiotics and prebiotics associated with aquaculture: a review. Fish Shellfish Immunol., **45**: 733-741.

AN, X., Q. BAO, S. DI, Y. ZHAO, S. ZHAO, H. ZHANG, F. LIAN & X. TONG, 2019. The interaction between the gut microbiota and herbal medicines. Biomed. Pharmacother., **118**: 109252.

ARISA, I. I., W. WIDANARNI, M. YUHANA, Z. A. MUCHLISIN & A. A. MUHAMMADAR, 2015. The application of probiotics, prebiotics and synbiotics to enhance the immune responses of vannamei shrimp (*Litopenaeus vannamei*) to *Vibrio harveyi* infection. Aquacult. Aquarium Conserv. Legislat., **8**: 772-778.

ASTUTI, D., K. BECKER & N. RICHTER, 2007. Utilization of methanol extracted of moringa and mulberry leaves to evaluate energy and protein balance of Nile tilapia. In: B. PONJO, PRIOSOERYANTO & R. TURIA (eds.), Empowering of society through the animal health and production activities with the appreciation to the indigenous knowledge: 70-82. (German Institute for Tropical and Subtropical Agriculture (DITSL GmbH), Manado).

BOONANUNTANASARN, S., U. WONGSASAK, T. PITAKSONG & S. CHAIJAMRUS, 2016. Effects of dietary supplementation with β-glucan and synbiotics on growth, haemolymph chemistry, and intestinal microbiota and morphology in the *Pacific white* shrimp. Aquacult. Nutr., **22**: 837-845.

CHEN, L., W. C. S. TAI & W. L. W. HSIAO, 2015. Dietary saponins from four popular herbal tea exert prebiotic-like effects on gut microbiota in C57BL/6 mice. J. Funct. Foods, **17**: 892-902.

EL-SAADONY, M. T., A. M. SHEHATA, M. ALAGAWANY, A.-M. E. ABDEL-MONEIM, D. A. SELIM, M. ABDO, A. F. KHAFAGA, K. A. EL-TARABILY, N. A. EL-SHALL & M. E. ABD EL-HACK, 2022. A review of shrimp aquaculture and factors affecting the gut microbiome. Aquacult. Int., **30**: 2847-2869.

ELABD, E. M. Y., S. M. MORSY & H. A. ELMALT, 2018. Investigating of *Moringa oleifera* role on gut microbiota composition and inflammation associated with obesity following high fat diet feeding. Open Access Maced. J. Med. Sci., **6**: 1359.

FIGUEREDO, A., C. LIRA, J. VERA-CARIPE, M. DE DONATO & C. LODEIROS, 2023. The Pacific white shrimp, the most cultivated shrimp species, is it *Litopenaeus* or *Penaeus vannamei*?. Rev. Aquacult., **15**: 7-13.

FILLY, A., T. FABIANO, S. ANNE, C. LOUIS, X. FERNANDEZ & F. CHEMAT, 2016. Water as a green solvent combined with different techniques for extraction of essential oil from lavender flowers. C. R. Chim., **19**: 707-717.

FOIDL, N., H. MAKKAR & K. BECKER, 2002. The potential of *Moringa oleifera* for agricultural and industrial uses. Development Potential for Moringa Products International workshop, 29th October-2nd November 2001, Dar es Salaam: 23-35.

FUANDILA, N. N., W. WIDANARNI & M. YUHANA, 2020. Growth performance and immune response of prebiotic honey fed Pacific white shrimp *Litopenaeus vannamei* to *Vibrio parahaemolyticus* infection. J. Appl. Aquacult., **32**: 221-235.

GAWAD, E. E. A. A. E., A. M. EL ASELY, E. I. SOROR, A. A. ABBASS & B. AUSTIN, 2020. Effect of dietary *Moringa oleifera* leaf on the immune response and control of *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*) fry. Aquacult. Int., **28**: 389-402.

GBADEBO, A., O. OKAREH, A. OGUNJOBI & A. DADA, 2019. Effects of *Moringa oleifera* lam. leaf powder on *Bifido acteria* and *Escherina coli* in the gut of albino rats. J. Adv. Microbiol., **18**: 1-11.

GIBSON, G. R., R. HUTKINS, M. E. SANDERS, S. L. PRESCOTT, R. A. REIMER, S. J. SALMINEN, K. SCOTT, C. STANTON, K. S. SWANSON, P. D. CANI, K. VERBEKE & G. REID, 2017. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Rev. Gastroenterol. Hepatol., **14**: 491-502.

HAFSA, A. S. H., S. A. IBRAHIM, Y. Z. EID & A. A. HASSAN, 2019. Effect of dietary *Moringa oleifera* leaves on the performance, ileal microbiota and antioxidative status of broiler chickens. J. Anim. Physiol. Anim. Nutr., **104**: 529-538.

HAMED, H. S. & Y. S. E. SAYED, 2019. Antioxidant activities of *Moringa oleifera* leaf extract against pendimethalin-induced oxidative stress and genotoxicity in Nile tilapia, *Oreochromis niloticus* (L.). Fish Physiol. Biochem., **45**: 71-82.

HELRICH, K., 1990. Official methods of analysis of the Association of Official Analytical Chemists. (Association of Official Analytical Chemists, Rockville, MD, USA).

HUEBNER, J., R. L. WEHLING & R. W. HUTKINS, 2007. Functional activity of commercial prebiotics. Int. Dairy J., **17**: 770-775.

IDRIS, A. & U. ABUBAKAR, 2016. Phytochemical and antibacterial investigations of moringa (*Moringa oleifera*) leaf extract on selected bacterial pathogens. J. Microbiol. Antimicrob., **8**: 28-33.

IMAIZUMI, K., S. TINWONGGER, H. KONDO & I. HIRONO, 2021. Analysis of microbiota in the stomach and midgut of two penaeid shrimps during probiotic feeding. Sci. Rep., **11**: 9936.

KALEO, I. V., Q. GAO, B. LIU, C. SUN, Q. ZHOU, H. ZHANG, F. SHAN, Z. XIONG, L. BO & C. SONG, 2019. Effects of *Moringa oleifera* leaf extract on growth performance, physiological and immune response, and related immune gene expression of *Macrobrachium rosenbergii* with *Vibrio anguillarum* and ammonia stress. Fish Shellfish Immunol., **89**: 603-613.

LI, C., Z. DONG, B. ZHANG, Q. HUANG, G. LIU & X. FU, 2020. Structural characterization and immune enhancement activity of a novel polysaccharide from *Moringa oleifera* leaves. Carbohydr. Polym., **234**: 115897.

LI, C., S. ZHOU, X. FU, Q. HUANG & Q. CHEN, 2021. In vitro digestibility and prebiotic activities of a bioactive polysaccharide from *Moringa oleifera* leaves. J. Food. Biochem., **45**: e13944.

LIU, D., X. H. DONG, B. P. TAN, Q. H. YANG, S. Y. CHI, H. Y. LIU & S. ZHANG, 2016. Effects of *Lactobacillus acidophilus* on growth performance, immunity and disease resistance in Pacific white leg shrimp *Litopenaeus vannamei*. Fish Sci., **35**: 37-42.

MAHMOOD, K. T., T. MUGAL & I. U. HAQ, 2010. *Moringa oleifera*: a natural gift — a review. J. Pharm. Sci., **2**: 775-781.

MALLIK, B. P. & H. BHAWSAR, 2018. Evaluation of prebiotic score of edible mushroom extract. Int. J. Eng. Res. Technol., **7**: IJERTV7IS100028.

MANAN, H. & M. IKHWANUDDIN, 2021. Triploid induction in penaeid shrimps aquaculture: a review. Rev. Aquacult., **13**: 619-631.

MILUTINOVIĆ, M., S. DIMITRIJEVIĆ-BRANKOVIĆ & M. RAJILIĆ-STOJANOVIĆ, 2021. Plant extracts rich in polyphenols as potent modulators in the growth of probiotic and pathogenic intestinal microorganisms. Front. Nutr., **8**: 688843.

MOGANA, R., A. ADHIKARI, M. N. TZAR, R. RAMLIZA & C. WIART, 2020. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. BMC Complement. Med. Ther., **20**: 55.

MORTON, J. F., 1991. The horseradish tree, *Moringa pterygosperma* (Moringaceae) — a boon to arid lands? Econ. Bot., **45**: 318-333.

MOYO, B., P. J. MASIKA & V. MUCHENJE, 2012. Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. Afr. J. Biotechnol., **11**: 2797-2802.

MUNAENI, W., WIDANARNI, M. YUHANA, M. SETIAWATI & A. T. WAHYUDI, 2020. Effect in white shrimp *Litopenaeus vannamei* of *Eleutherine bulbosa* (Mill.) Urb. powder on immune genes expression and resistance against *Vibrio parahaemolyticus* infection. Fish Shellfish Immunol., **102**: 218-227.

NANASOMBAT, S., N. KUNCHAROEN, B. RITCHAROON & P. SUKCHAROEN, 2018. Antibacterial activity of Thai medicinal plant extracts against oral and gastrointestinal pathogenic bacteria and prebiotic effect on the growth of *Lactobacillus acidophilus*. Chiang Mai J. Sci., **45**: 33-34.

NOWAK, R., N. NOWACKA-JECHALKE, M. JUDA & A. MALM, 2018. The preliminary study of prebiotic potential of Polish wild mushroom polysaccharides: the stimulation effect on *Lactobacillus* strains growth. Eur. J. Nutr., **57**: 1511-1521.

ORDAZ, P. R., M. A. WALL, M. G. GOÑI, C. M. G. RAMOS, Z. J. F. AYALA & A. G. A. GONZÁLEZ, 2018. Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. Lett. Appl. Microbiol., **66**: 25-31.

PATIL, P. K., T. N. VINAY, R. ARAVIND, S. AVUNJE & K. K. VIJAYAN, 2021. Effect of *Bacillus* spp. on the composition of gut microbiota in early life stages of Indian white shrimp, *Penaeus indicus*. J. Appl. Aquacult. DOI:10.1080/10454438.2021.2011527.

PRAYITNO, S. B., S. RIYADI, SARJITO & DESRINA, 2021. The performance of survival and immunity of catfish (*Clarias gariepinus*) juvenile infected by *Aeromonas hydrophila* after treated by prebiotic extract in the diet. J. Phys. Conf. Ser., **1943**: 012083.

RICHTER, N., P. SIDDHURAJU & K. BECKER, 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture, **217**: 599-611.

RINGO, E., R. E. OLSEN, T. Ø. GIFSTAD, R. A. DALMO, H. AMLUD, G. I. HEMRE & A. M. BAKKE, 2010. Prebiotics in aquaculture: a review. Aquacult. Nutr., **16**: 117-136.

SANTO, A. P., E. N. S. CARTOLANO, T. F. SILVA, F. A. S. M. SOARES, L. A. GIOIELLI, P. PEREGO, A. CONVERTI & M. N. OLIVEIRA, 2012. Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts. Int. J. Food Microbiol., **154**: 135-144.

SHERIF, A. H., A. M. EL-GAMAL & A. E. TOLAN, 2014. Incorporation of *Moringa oleifera* leaf in Nile tilapia *Oreochromis niloticus* diet and its effect on growth performance and immune status. J. Vet. Sci., **1**: 806-814.

THITIRATSAKUL, B. & P. ANPRUNG, 2014. Prebiotic activity score and bioactive compounds in longan (*Dimocarpus longan Lour.*): influence of pectinase in enzyme-assisted extraction. J. Food Sci. Technol., **51**: 1947-1955.

UNEGBU, V., N. NKWOEMEKA, F. OKEY NDECHE & C. OBUM NNADI, 2020. Phytochemical and antibacterial properties of *Moringa oleifera* leaf extracts on *Escherichia coli* and *Staphylococcus aureus*. Niger. J. Microbiol., **34**: 5145-5152.

VONGSAK, B., P. SITHISARN, S. MANGMOOL, S. THONGPRADITCHOTE, Y. WONGKRAJANG & W. GRITSANAPAN, 2013. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind. Crops Prod., **44**: 566-571.

WANG, F., Y. F. BAO, J. J. SI, Y. DUAN, Z. B. WENG & X. C. SHEN, 2019. The beneficial effects of a polysaccharide from *Moringa oleifera* leaf on gut microecology in mice. J. Med. Food, **22**: 907-918.

WANG, Y. & Q. GU, 2010. Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. Mar. Biol. Res., **6**: 327-332.

YOO, H. D., D. KIM & S. H. PAEK, 2012. Plant cell wall polysaccharides as potential resources for the development of novel prebiotics. Biomol. Ther. (Seoul), **20**: 371-379.

YU, M. C., Z. J. LI, H. Z. LIN, G. L. WEN & S. MA, 2009. Effects of dietary medicinal herbs and Bacillus on survival, growth, body composition, and digestive enzyme activity of the white shrimp *Litopenaeus vannamei*. Aquacult. Int., **17**: 377-384.

ZENG, P., 2019. Effect of dietary *Lactobacillus plantarum* and *Lactobacillus acidophilus* on water qualities, growth, and non-spesific immune responses of *Litopenaeus vannamei*, Aquaculture Department. National Taiwan Ocean University, Keelung.

ZOQRATT, M. Z. H. M., W. W. H. ENG, B. T. THAI, C. M. AUSTIN & H. M. GAN, 2018. Microbiome analysis of Pacific white shrimp gut and rearing water from Malaysia and Vietnam: implications for aquaculture research and management. PeerJ, **6**: 5826.

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