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Determination of optimum probiotic dosage for the culture of whiteleg shrimp, *Litopenaeus vannamei* in an indoor system

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Determination of probiotic dosages in BFT using Principal component analysis via contributory effect

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ABSTRACT

Determining the optimum application dosage of probiotic in biofloc system is often challenging because the microbial community seems to exert similar effects irrespective of their dosages. It is however noted that certain dosages promote higher yield in shrimp culture more effectively. Principal component analysis was adopted to identify these optimum dosages where 1-way ANOVA could not clearly identify due to the effects of microbial community. The effect of varying application dosages of probiotics in a shrimp culture system on growth indices and water quality variables were studied in a culture trial of *Litopenaeus vannamei* that lasted for 84 days in an indoor biofloc culture system. Only 4 (26.66 %) of variables showed significant difference in the 1-way ANOVA conducted. Principal Component Analysis (PCA) was used to illustrate the dimensional interactions among variables. Contributions of observation points at 7 $ml.l^{-1}$ (Obs1–3), 14 $ml.l^{-1}$ (Obs 4–6), and 21 $ml.l^{-1}$ (Obs 7–9) were analyzed. The Obs 4–6 (representing 14 $ml.l^{-1}$) had the highest mean contributory effect (11.03) indicating the greatest impact recorded in the PCA relationships among growth indices and water quality variables. It was concluded that probiotic dosages can be determined in biofloc system based on the contributory effect it enhances in the measured variables.

- Microbial community in biofloc affects the clear distinctions of the dosages due to proliferations
- 1-way ANOVA alone may not show dosage-related variations.
- PCA could identify the optimum probiotic dosage that would enhance shrimp growth performance and maintain favourable water quality conditions in biofloc aquaculture systems.

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Background

Identifying the optimum probiotic dosage in biofloc aquaculture systems is a complex task that entails a comprehensive understanding of multiple interrelating variables. Traditional statistical methods such as one-way Analysis of Variance (ANOVA) are widely used in aquaculture research to determine the significant differences between treatment groups, such as probiotic dosages (Vázquez-Euán et al., 2022; [1]). However, while one-way ANOVA can identify these statistical differences in specific parameters, it has limitations when applied to the dynamic and multifactorial nature of biofloc systems caused by the microbial community. A biofloc environment is influenced by several interdependent factors, such as water quality parameters (e.g., ammonia, nitrate, and pH), balanced microbial community, health indicators of shrimp, and growth performance indices [2]. Instead of these variables acting independently; they are intricately interconnected, where one parameter often influences others. One-way ANOVA, which analyzes each variable in isolation, is limited in its ability to capture these complex interactions. As a result, relying solely on ANOVA in inadequate in identifying the key patterns and relationships that could be critical to identifying the optimal probiotic level.

This limitation creates a need for multivariate techniques that can analyze multiple variables simultaneously to reveal underlying patterns and interactions. Principal Component Analysis (PCA) is a powerful statistical tool that addresses this need by reducing the dimensionality of the dataset while retaining the most important information [3,4]. By transforming the original variables into a set of uncorrelated components, PCA identifies the main factors influencing the variability within the system. This is particularly important in biofloc aquaculture, where the aim is not only to optimize individual parameters but also to maintain a stable microbial community and achieve balanced environmental conditions. PCA has several advantages over traditional univariate methods. It allows for the detection of latent structures among variables, aiding the choice of different probiotic dosages that affect water quality, changes in microbial communities, and shrimp health in a holistic manner. PCA also helps to visualize correlations between multiple variables and identify the key drivers of variability [5] in order to determine the culture conditions under varying probiotic levels.

This study aims to provide a better understanding of the interactions within the biofloc system by complementing one-way ANOVA with PCA. While ANOVA could not identify the optimum dosages of probiotic due to statistical insignificance across the dosages, PCA explained the multivariate relationships necessary for identifying the optimum probiotic dosage. These methods provide an evaluation of the biofloc system, by identifying the recommended dosage needed for improved growth of shrimp and long-term stability of the microbial environment.

Method details

The problem: Conventional approaches of determining these application dosages of probiotic employs the usage of one-way ANOVA. However, due to the complex interactions among microbial populations in BFT, it is difficult to obtain these clear variations in terms of dosages. Principal Component Analysis (PCA) presents a more advanced methodology which effectively show the complex relationships between various water quality parameters. By reducing dimensionality and revealing the influential variables, PCA aids in identifying observation points with the highest impact. In this way, the application dosage constituting the best dimensional effect could be identified by a principal component analysis. This experiment investigated the application dosage of Rapid biofloc^{TM} (a pure culture of *Bacillus tropicus*) that produced the optimum growth response of *Litopenaeus vannamei* and water quality conditions in a zero-water discharge biofloc condition which was undeterminable using one-way ANOVA.

Probiotics in biofloc aquaculture system

The application dosage of probiotics directly influences the microbial composition and activity within the biofloc system, which affects the overall water quality, including the levels of ammonia, nitrites, nitrates, and other key parameters necessary for the health and growth of aquatic organisms [6]. Identifying the optimal dosage of probiotics is essential for maintaining a stable and healthy environment within the biofloc system, which directly impacts the productivity of cultured aquatic species [7,8]. Microbial communities in biofloc system stimulate the growth response and water quality conditions irrespective of application dosages as long as a community is established by the inoculum (Shitu, 2021). This stimulation often indicates similarities in the variable responses recorded in such conditions. There is a need to assess the minute dimensional effects the measured variables have on each other in response to the varying dosages administered to the system so as to evaluate the impact of application dosages of probiotic in a biofloc system.

Plate 1. Indoor biofloc aquaculture system.

Experimental setup

This experiment was conducted at the Institute of Tropical Aquaculture and Fisheries, AKUATROP, Universiti Malaysia Terengganu, for 84 days (14th March – 5th June, 20223). Stocking was done at 200 individuals per m^3 (average weight, 0.91 g) in $3m^3$-HDPE tanks, and feeding was administered to satiation three times daily (Plate 1). Pre-measured feed (36 % crude protein) weighing 100 g were taken for each tank and to determine the amount consumed, the respective weights of the remaining feed was once again taken after feeding for each tank. Molasses was applied as a carbon source to maintain the carbon-to-nitrogen level at C15N. The amount of molasses was measured from the amount of feed administered and in reference to the crude protein level described by Minabi (2020). The water quality was routinely measured every week during the culture period to evaluate the optimum performance of the microbial community in the culture systems.

The procedure of bacteria culture began with the cultivation of actively growing cells of Bacillus tropicus for 18 h, followed by the selection of approximately three distinct colonies, which were then transferred into a clean test tube containing approximately 5–9 mL of physiological saline. The vortexing process was conducted, and the turbidity was assessed in relation to the No 5 MacFarland standard to determine the bacterial stock concentration. Subsequently, serial dilution was carried out, with each dilution vortexed for approximately 10 s to ensure homogeneity, which was achieved by adding 1000 µL of stock to 9000 µL of sterile physiological saline. Plating 100 µL from the fifth dilution was then performed, followed by incubation at 25 ± 2 °C for 18–24 h. Colonies were counted, and the resulting numbers were multiplied by the corresponding dilution factors

$$CFU/\text{mL} = colony\ count\ X\ \left(10^{-5}\right)\ X\ 100$$

The population of the heterotrophic bacteria counted in 1 mL were in the range of 80–120 × 10^6 CFU/mL. These predetermined bacteria counted were then varied in population by applying 7, 14 and 21 $ml.l^{-1}$ to 3 tanks each of the culture tanks.

Water quality and growth monitoring

Water quality was monitored weekly during the culture period. In this study, water quality parameters were meticulously assessed to ensure the precise measurement of various crucial factors that impact aquaculture systems. A multiparameter water checker, YSI Professional, was used to measure temperature (Temp), dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity

(EC), pH, and salinity (Sal). Established standard procedures were followed to ensure accuracy and consistency in these chemical analyses. In addition, total ammonia nitrogen (TAN), nitrite (NO_2^-), and phosphate (PO_4^{3-}) were analyzed using a spectrophotometer, Shimadzu UV-1800 model. The method used for TAN measurement adhered to the procedures of Liang et al. [9]. For the assessment of NO_2^- levels, the methodology outlined in Lin et al. [10] was methodically followed, while PO_4^{3-} concentrations were determined following the protocols described by Shyla et al. [11]. The floc level was measured with an Imhoff cone.

A sample of shrimps was randomly selected, and their initial weights (W_1) and lengths (L_1) were recorded. The final individual weights (W_2) and lengths (L_2) were measured at the end of the feeding period. Key growth performance indices such as mean weight gain (MWG), specific growth rate (SGR), food conversion ratio (FCR) indicating feeding efficiency, condition factor (CF), and percentage survival (% surv) were measured from the relationships in Eqs. (1)–(5).

The mean weight gain, MWG was calculated from the relationship

$$MWG\ (g) = \frac{(W2 - W1)}{Culture\ period\ (84\ days)} \tag{1}$$

The specific growth rate, SGR was calculated from the relationship

$$SGR\ (g/day) = \left[\frac{(\ln W2 - \ln W1)}{culture}\right] X\ 100 \tag{2}$$

Feed conversion ratio, FCR was computed from the equation

$$FCR = \frac{Quantity\ of\ feed\ administered\ (g)}{Weight\ gain\ (g)} \tag{3}$$

The mean condition factor of the shrimp was calculated from the relationship

$$CF = \frac{100 \times W2}{L2 \times 3} \tag{4}$$

The percentage survival was calculated from the equation

$$\%\ Survival = \left[\frac{number\ of\ shrimp\ at\ harvest}{number\ of\ shrimp\ at\ stocking}\right] X\ 100 \tag{5}$$

Statistical analysis

Data obtained from water quality monitoring and derived growth indices from body measurements were subjected to principal component analysis on XLSTAT version 2018.1 for Windows. Principal Component Analysis (PCA) was employed to investigate the relationships between water quality parameters and growth indices in the indoor biofloc aquaculture. Also, PCA was used to determine the minute influences the zootechnical and water quality variables had on each other since the variations between application dosages did not show marked differences. The PCA multivariate statistical technique reduced the dimensionality of the two datasets by identifying underlying patterns and correlations and simplified the complex interplay between water quality factors and growth indices. Accordingly, the analyses illustrated the variations in water quality and their impacts on the growth performance of the cultured shrimp.

The summary results of the data obtained for the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system during the experiment are presented in Table 1.

Statistical analysis using 1-Way ANOVA shows that 3 (MWG, SGR and % surv) did not vary across the application dosages. Similarly, TAN, PO4, Temp, DO, EC, TDS, sal, and pH did not change with probiotic application dosages for the water quality variables. Condition Factor (CF) and Feed Conversion Ratio (FCR), however, showed a significant difference ($p < 0.05$) with the application dosages of probiotic. The values for CF were highest (0.98) at 21 $ml.l^{-1}$ while FCR was highest (1.41) at 7 $ml.l^{-1}$ dosage. Among the water quality parameters, Nitrite (NO_2^-) and floc Level significantly differed across the application dosages. Generally, only 4 variables (26.66 %) varied with the application dosages.

Table 2 shows Pearson correlations of growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system. MWG showed significant positive correlations with several parameters, including CF, SGR, DO, EC, TDS, Sal, and pH. Additionally, CF was positively correlated with SGR, DO, and Sal while negatively correlated with FCR, Temp, and TAN. SGR, a crucial growth indicator, showed positive correlations with DO and EC but negative correlations with FCR and TAN. % Surv was positively related to Temp and Sal but negatively associated with FCR, TAN, NO_2^- and pH. The FCR was significantly positively correlated with Temp, TDS, and Sal but negatively correlated with MWG, CF, SGR, TAN, and NO_2^-. TAN displayed positive correlations with EC and Sal while being negatively correlated with FCR and MWG. NO_2^- had positive correlations with PO_4^{3-} and EC, but it negatively correlated with % surv and MWG. PO_4^{3-} was positively correlated with Temp, DO, and Sal but negatively correlated with FCR and MWG. Temp exhibited positive correlations with DO, EC, and Sal but negative correlations with MWG, CF, and FCR. DO was positively correlated with EC and TDS but negatively correlated with MWG, CF, SGR, Temp, and FCR. EC showed positive correlations with TDS and Sal but negative correlations with MWG, SGR, and Temp. TDS was positively correlated with Sal but negatively correlated with MWG, FCR, and Temp. Sal demonstrated a positive correlation with pH but negative correlations with MWG, CF, SGR, % surv, and FCR. Finally, pH had a strong negative correlation with NO_2^- and a significant negative correlation with Floc level, while Floc level showed a positive correlation with NO_2^-.

Table 1
Growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variable	Application dosage (ml.l^{-1})			*p*-value
	7	14	21	
Growth indices				
MWG (g)	7.05 ± 0.63	8.83 ± 0.01	8.28 ± 0.96	0.48ns
CF	0.85 ± 0.02^{b}	0.96 ± 0.01^{ab}	0.98 ± 0.05^{a}	0.04
SGR (g.day^{-1})	4.22 ± 0.11	4.52 ± 0.11	4.48 ± 0.16	0.30ns
% Surv	49.30 ± 0.45	59.78 ± 0.88	48.03 ± 0.81	0.14ns
FCR	1.41 ± 0.07^{a}	1.13 ± 0.06^{b}	1.11 ± 0.02^{b}	0.02
Water quality				
TAN (mg.l^{-1})	1.02 ± 0.01	1.01 ± 0.02	1.15 ± 0.07	0.17ns
NO_2^- (mg.l^{-1})	0.46 ± 0.03^{b}	0.40 ± 0.02^{b}	0.50 ± 0.02^{a}	0.04
PO_4^{3-}	0.13 ± 0.05	0.13 ± 0.01	0.12 ± 0.00	0.19ns
Temp (°C)	28.64 ± 0.05	28.66 ± 0.02	28.68 ± 0.01	0.12ns
DO (mg.l^{-1})	5.61 ± 0.09	5.61 ± 0.15	5.62 ± 0.14	0.99ns
EC (µS.cm^{-1})	47,682.00 ± 766	47,746.00 ± 800	46,691.00 ± 640	0.55ns
TDS (mg.l^{-1})	27,323.00 ± 599	28,822.00 ± 174	28,807.00 ± 893	0.23ns
Sal (ppm)	34.05 ± 0.14^{b}	35.13 ± 0.11^{a}	35.02 ± 0.23^{a}	0.12ns
pH	7.50 ± 0.04	7.56 ± 0.02	7.46 ± 0.05	0.35ns
Floc level (ml.l^{-1})	1.14 ± 0.08^{b}	2.74 ± 0.90^{a}	1.93 ± 0.24^{b}	0.03

ns=not significant.

Table 2
Pearson correlations of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variables	MWG	CF	SGR	% Surv	FCR	TAN	NO_2^-	PO_4^{3-}	Temp	DO	EC	TDS	Sal	pH	Floc level
MWG	1	0.753	0.968	0.459	−0.777	−0.201	−0.171	−0.121	−0.228	0.531	0.662	0.750	0.422	0.058	0.060
CF	0.753	1	0.798	0.255	−0.817	0.147	0.009	−0.164	−0.534	0.303	0.042	0.911	0.798	0.193	0.268
SGR	0.968	0.798	1	0.447	−0.811	−0.178	−0.039	−0.137	−0.279	0.447	0.592	0.786	0.489	−0.011	0.187
% surv	0.459	0.255	0.447	1	−0.296	−0.471	−0.444	−0.002	−0.340	0.320	0.372	0.101	0.305	0.323	−0.093
FCR	−0.777	−0.817	−0.811	−0.296	1	−0.323	−0.022	0.382	0.521	−0.097	−0.226	−0.771	−0.703	0.097	−0.462
TAN	−0.201	0.147	−0.178	−0.471	−0.323	1	0.303	−0.495	−0.241	0.289	−0.514	0.090	0.288	−0.186	0.378
NO_2^-	−0.171	0.009	−0.039	−0.444	−0.022	0.303	1	0.045	0.453	0.463	−0.128	0.007	−0.303	−0.812	0.544
PO_4^{3-}	−0.121	−0.164	−0.137	−0.002	0.382	−0.495	0.045	1	0.579	0.324	0.229	0.112	−0.329	0.177	−0.627
Temp	−0.228	−0.534	−0.279	−0.340	0.521	−0.241	0.453	0.579	1	0.212	0.410	−0.327	−0.895	−0.455	−0.351
DO	−0.531	−0.303	−0.447	−0.320	0.097	0.289	0.463	0.324	0.212	1	−0.357	−0.147	−0.143	−0.419	0.270
EC	0.662	0.042	0.592	0.372	−0.226	−0.514	−0.128	0.229	0.410	−0.357	1	0.202	−0.309	−0.159	−0.294
TDS	0.750	0.911	0.786	0.101	−0.771	0.090	0.007	0.112	−0.327	−0.147	0.202	1	0.679	0.190	0.066
Sal	0.422	0.798	0.489	0.305	−0.703	0.288	−0.303	−0.329	−0.895	−0.143	−0.309	0.679	1	0.467	0.262
pH	0.058	0.193	−0.011	0.323	0.097	-0.186	−0.812	0.177	−0.455	−0.419	−0.159	0.190	0.467	1	−0.655
Floc level	0.060	0.268	0.187	−0.093	−0.462	0.378	0.544	-0.627	−0.351	0.270	−0.294	0.066	0.262	−0.655	1

Values in bold are different from 0 with a significance level α=0.95.

Table 3
Eigenvalues of the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	5.678	3.405	2.625	1.474	0.931	0.538	0.294	0.055
Variability (%)	37.852	22.699	17.498	9.825	6.207	3.588	1.962	0.368
Cumulative %	37.852	60.551	78.049	87.874	94.081	97.670	99.632	100.000

Table 1 presents the descriptive statistics and ANOVA results for various growth indices and water quality parameters at different probiotic dosages. While the 1-way ANOVA showed limited statistical significance across dosages, the Pearson correlation matrix (Table 2) indicated the interconnected level of influences of the growth indices with the water quality variables. MWG and SGR had strong positive correlations with dissolved oxygen (DO), electrical conductivity (EC) and total dissolved solids (TDS) indicating positive impact of these water conditions in support of optimum growth. This trend was further supported by the FCR having negative correlations with the same water quality variables whereby optimum growth is indicated by lower FCR. Conversely, total ammonia-nitrogen (TAN), nitrite (NO_2^-) and phosphate (PO_4^{3-}) all had negative correlations with the growth parameters, explaining their unfavorable impacts on growth. These observations suggested the need for multivariate techniques (principal component analysis) to further reveal these complex relationships, as ANOVA alone could not capture dosage-related variations effectively.

Principal Component Analysis (PCA) was employed to explain the multidimensional relationships between growth indices and water quality parameters, as shown by the Eigenvalues in Table 3. Factor 1 (F1) and Factor 2 (F2) accounted for 60.55 % of the total

Table 4
Eigenvectors of the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variables	F1	F2	F3	F4	F5	F6	F7	F8
Growth indices								
MWG (g)	0.364	−0.135	−0.246	−0.065	−0.113	0.063	−0.051	−0.329
CF	0.382	0.089	−0.041	0.226	−0.055	−0.288	0.188	−0.390
SGR ($g.day^{-1}$)	0.372	−0.073	−0.263	−0.039	−0.015	−0.057	−0.043	0.425
% Surv	0.201	−0.253	0.050	−0.255	0.597	0.121	0.609	−0.072
FCR	−0.369	−0.174	0.132	−0.019	−0.049	−0.366	0.022	0.163
Water quality								
TAN ($mg.l^{-1}$)	0.016	0.423	0.123	0.099	−0.386	0.506	0.444	0.020
NO_2^- ($mg.l^{-1}$)	−0.112	0.328	−0.393	0.126	0.026	−0.361	0.424	0.428
PO_4^{3-} ($mg.l^{-1}$)	−0.132	−0.292	−0.154	0.579	0.236	−0.063	0.041	−0.081
Temp (°C)	−0.274	−0.150	−0.400	0.119	−0.191	0.043	0.238	−0.258
DO ($mg.l^{-1}$)	−0.189	0.260	−0.079	0.369	0.508	0.421	−0.224	0.018
EC ($\mu S.cm^{-1}$)	0.104	−0.328	−0.408	−0.179	−0.072	0.365	−0.144	0.336
TDS ($mg.l^{-1}$)	0.342	0.022	−0.126	0.428	−0.115	−0.059	−0.146	0.017
Sal (ppm)	0.341	0.140	0.279	0.176	0.136	−0.029	−0.029	0.322
pH	0.115	−0.290	0.461	0.213	−0.099	−0.028	0.107	0.167
Floc level ($ml.l^{-1}$)	0.091	0.447	−0.128	−0.275	0.281	−0.232	−0.220	−0.160

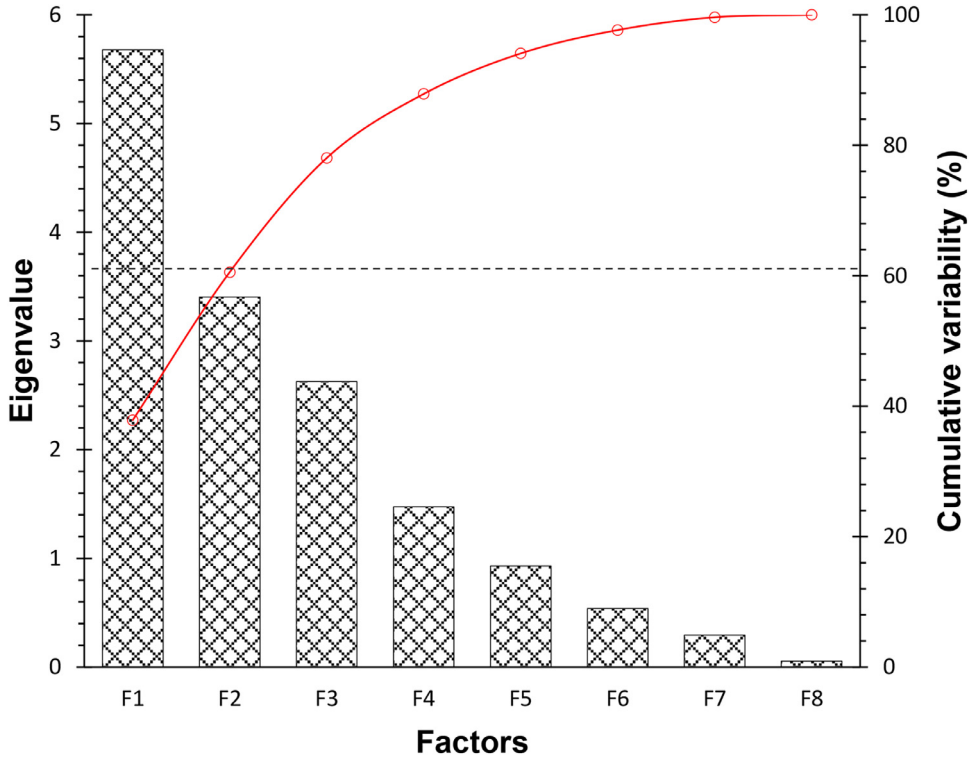

Fig. 1. Eigenvalues and cumulative variability (%) of PCA of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

variance, where F1 captured growth-related variables and F2 water quality variables respectively. Stepwise, Table 4 presented the corresponding eigenvectors, with high loadings of MWG, CF, and SGR in F1 and TAN and NO2- in F2. This reduction in dimensionality illustrated the interactions between the growth performance and water quality. The eigenvalues of the PCA conducted on the variables are presented in Table 3. The table shows the eigenvalues for eight factors, F1 to F8. The first factor (F1) had the highest eigenvalue (5.678), followed by F2 (3.405) and F3 (2.625). Also, F1 had the highest variability (37.852 %) in the data, while F2 recorded 22.699 % and F3 (17.498 %).

Further analyses were concentrated on factors F1 and F2 because the cumulative variability (60.551 %) accounted for more than 50% of the correlations and variabilities of the variables analyzed in the PCA (Fig. 1).

Table 5
Factor loadings of the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variables	F1	F2	F3	F4	F5	F6	F7	F8
Growth indices								
MWG (g)	0.867	−0.250	−0.398	−0.078	−0.109	0.046	−0.028	−0.077
CF	0.909	0.165	−0.067	0.274	−0.053	−0.211	0.102	−0.092
SGR ($g.day^{-1}$)	0.886	−0.135	−0.426	−0.047	−0.015	−0.042	−0.023	0.100
% Surv	0.480	−0.468	0.080	−0.310	0.576	0.088	0.331	−0.017
FCR	−0.880	−0.321	0.214	−0.023	−0.048	−0.268	0.012	0.038
Water quality								
TAN ($mg.l^{-1}$)	0.037	0.781	0.199	0.120	−0.373	0.371	0.241	0.005
NO_2^- ($mg.l^{-1}$)	−0.267	0.605	−0.636	0.152	0.025	−0.265	0.230	0.101
PO_4^{3-} ($mg.l^{-1}$)	−0.315	−0.539	−0.249	0.702	0.228	−0.047	0.022	−0.019
Temp (°C)	−0.653	-−0.277	−0.649	0.145	−0.184	0.031	0.129	−0.061
DO ($mg.l^{-1}$)	−0.450	0.480	−0.127	0.448	0.490	0.309	−0.121	0.004
EC ($\mu S.cm^{-1}$)	0.247	−0.605	−0.661	−0.217	−0.070	0.268	−0.078	0.079
TDS ($mg.l^{-1}$)	0.816	0.041	−0.205	0.520	−0.111	−0.043	−0.079	0.004
Sal (ppm)	0.812	0.259	0.452	0.214	0.131	−0.022	−0.016	0.076
pH	0.273	−0.535	0.747	0.259	−0.095	−0.021	0.058	0.039
Floc level ($ml.l^{-1}$)	0.216	0.825	−0.208	−0.334	0.271	−0.170	−0.120	−0.038

Table 6
Correlations between variables and factors of the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variables	F1	F2	F3	F4	F5	F6	F7	F8
Growth indices								
MWG (g)	0.867	−0.250	−0.398	−0.078	−0.109	0.046	−0.028	−0.077
CF	0.909	0.165	−0.067	0.274	−0.053	−0.211	0.102	−0.092
SGR ($g.day^{-1}$)	0.886	−0.135	−0.426	−0.047	−0.015	−0.042	−0.023	0.100
% Surv	0.480	−0.468	0.080	−0.310	0.576	0.088	0.331	−0.017
FCR	−0.880	−0.321	0.214	−0.023	−0.048	−0.268	0.012	0.038
Water quality								
TAN ($mg.l^{-1}$)	0.037	0.781	0.199	0.120	−0.373	0.371	0.241	0.005
NO_2^- ($mg.l^{-1}$)	−0.267	0.605	−0.636	0.152	0.025	−0.265	0.230	0.101
PO_4^{3-} ($mg.l^{-1}$)	−0.315	−0.539	−0.249	0.702	0.228	−0.047	0.022	−0.019
Temp (°C)	−0.653	−0.277	−0.649	0.145	−0.184	0.031	0.129	−0.061
DO ($mg.l^{-1}$)	−0.450	0.480	−0.127	0.448	0.490	0.309	−0.121	0.004
EC ($\mu S.cm^{-1}$)	0.247	−0.605	−0.661	−0.217	−0.070	0.268	−0.078	0.079
TDS ($mg.l^{-1}$)	0.816	0.041	−0.205	0.520	−0.111	−0.043	−0.079	0.004
Sal (ppm)	0.812	0.259	0.452	0.214	0.131	−0.022	−0.016	0.076
pH	0.273	−0.535	0.747	0.259	−0.095	−0.021	0.058	0.039
Floc level ($ml.l^{-1}$)	0.216	0.825	−0.208	−0.334	0.271	−0.170	−0.120	−0.038

The eigenvectors obtained from PCA are presented in Table 4. Eigenvectors represent the direction or orientation of the principal components (or factors) in the original data space. Each eigenvector corresponds to a specific principal component, linear combinations of the original variables whose directions are determined by the eigenvectors. The first principal component (F1) corresponded with the eigenvector with the highest eigenvalue and representing the direction of maximum variability in the data.

F1 had positive eigenvectors for MWG (0.364), CF (0.382), SGR (0.372), % Surv (0.201), TAN (0.016), EC (0.104), TDS (0.342), Sal (0.341), and pH (0.115). It also had negative eigenvectors for FCR (−0.369), NO_2^- (−0.112), PO_4^{3-} (−0.132), Temp (−0.274), DO (−0.189), and floc level (0.091). F1 represented a combination of variables from both growth indices and water quality. F2 has positive eigenvectors for CF (0.089), TAN (0.423), NO_2^- (0.328), DO (0.260), TDS (0.022), Sal (0.140), pH (−0.290), and floc level (0.447) while MWG (−0.135), SGR (−0.073), % Surv (−0.253), FCR (−0.174), Temp (−0.150), EC (−0.328) had negative eigenvectors.

Table 5 presents the factor loadings of PCA of the dataset obtained in the experiment. Factor loadings represent the relationships between the original variables and the principal components (factors) extracted from the data thus providing an understanding of the contributions of each original variable to the principal components and the variability in the data. The factor loadings (Table 5), correlations between variables and factors of the principal component analysis (Table 6) and contribution of variables (Table 7) further showed the specific contributions of growth indices and water quality parameters within each factor. MWG, CF, SGR and FCR had high contributions in F1, representing their roles in growth dynamics, while TAN and NO_2^-, EC and floc level contributed significantly to F2, suggesting their importance in maintaining water quality.

The high positive loadings for MWG (0.867), CF (0.909), SGR (0.886), and negative loadings for FCR (-0.880) suggest that F1 was primarily associated with growth-related variables. The high positive loadings for water quality variables (TAN, NO_2^-, PO_4^{3-}, Temp, DO, EC, TDS, Sal, pH, Floc level) in F2 +shows its direct association with water quality parameters. FCR was seen to have an opposite relationship with the other growth indices to illustrate its trend and preferences at lower numerical values. Factors 3–8 also

Table 7
Contribution of variables in the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variables	F1	F2	F3	F4	F5	F6	F7	F8
Growth indices								
MWG (g)	13.253	1.829	6.033	0.418	1.271	0.394	0.265	10.846
CF	14.568	0.796	0.169	5.094	0.305	8.277	3.549	15.193
SGR ($g.day^{-1}$)	13.837	0.533	6.914	0.149	0.024	0.331	0.187	18.043
% Surv	4.055	6.419	0.246	6.509	35.593	1.454	37.149	0.511
FCR	13.641	3.035	1.740	0.035	0.243	13.392	0.050	2.666
Water quality								
TAN ($mg.l^{-1}$)	0.024	17.923	1.508	0.973	14.904	25.596	19.678	0.038
NO_2^- ($mg.l^{-1}$)	1.260	10.762	15.429	1.575	0.067	13.021	18.005	18.347
PO_4^{3-} ($mg.l^{-1}$)	1.749	8.517	2.371	33.477	5.592	0.402	0.171	0.664
Temp (°C)	7.507	2.252	16.034	1.428	3.644	0.184	5.655	6.634
DO ($mg.l^{-1}$)	3.566	6.760	0.619	13.601	25.809	17.759	5.007	0.031
EC ($\mu S.cm^{-1}$)	1.079	10.765	16.624	3.198	0.524	13.297	2.072	11.270
TDS ($mg.l^{-1}$)	11.720	0.049	1.600	18.342	1.334	0.346	2.127	0.029
Sal (ppm)	11.601	1.970	7.801	3.112	1.844	0.086	0.084	10.395
pH	1.316	8.397	21.262	4.538	0.976	0.078	1.150	2.773
Floc level ($ml.l^{-1}$)	0.824	19.992	1.650	7.551	7.871	5.381	4.853	2.558

represented a combination of growth-related and water quality variables, with varying patterns of loadings which could have inert impact on the growth of the shrimp.

Additionally, Table 6 presents the correlations between variables and factors in the PCA. Variables with low correlations with the factors were considered less important in the PCA to reduce the variability in the dataset.

The established trend in the correlations confirmed that growth indices (MWG, CF, SGR, % Surv, FCR) exhibited strong positive correlations with Factor 1 (F1) showing its clear identity of growth-related parameters. On the other hand, water quality variables (TAN, NO_2^-, PO_4^{3-}, Temp, DO, EC, TDS, Sal, pH, Floc level), showed strong positive correlations with Factor 2 (F2), signifying its primary representation of water quality. The other factors (F3 to F8) capture complex combinations of growth indices and water quality variables, suggesting interactions between the two categories.

Furthermore, Fig. 2 illustrates the spatial positions of the variables based on their correlations.

All the growth indices had strong correlations (>0.5) in the F1 factor, which had the highest influence (37.85 %) in the PCA. MWG had a correlation coefficient of 0.87, SGR 0.88, and CF 0.90. % Surv, however, had weak correlations (0.47) in the F1 factor. The correlation coefficient of FCR was -0.88, indicating an inverse relationship established in its preference. Temp, TDS, Sal, and DO had weak correlations (<0.05) in the F2 factor, whose percentage contribution to the PCA was 22.70 %. On the other hand, floc level, TAN, NO_2^-, PO_4^{3-}, EC, and pH, had strong correlations depicting their influence on the entire water quality conditions of the culture system.

Table 7 presents the contribution level of each of the variables in the PCA. PCA aims to reduce the dimensionality of a dataset by transforming the original variables into a smaller set of uncorrelated principal components [12,13]. Variables with higher contributions were considered more important in representing the dataset. In this way, variables that contributed significantly to a specific principal component indicated the aspects of the data the component defined.

CF (14.568 %) and MWG (13.253 %) had substantial contributions in F1. These two parameters were considered in this experiment to have the foremost impact on the growth and conditions of well-being of the shrimp in the culture system. In contrast, the other growth indices had contributions ranging from 0.169 % to 6.914 %. TAN (17.923 %) and NO_2^- (10.76 %) contributed most significantly to F2.

The squared cosines of the variables are presented in Table 8. Squared cosines are measured to evaluate the representativeness of each variable by the principal components [14]. High squared cosines indicated that the variations of a variable were adequately explained by the components and the relationships that were established between variables and factor components. The squared cosines of the variables provided a quantitative measure of how each variable related to the principal components in the Principal Component Analysis (PCA) [15]. Squared cosines are between 0 and 1, where 1 indicates that the respective principal component entirely explains the variations of a variable, and 0 signifies that the variations of the variable are orthogonal (uncorrelated) to the component.

CF and MWG had high squared cosines of 0.827 and 0.752, respectively, in F1, indicating that a substantial proportion of their variation aligned with the first principal component, while in F2, TAN and NO2 exhibited high squared cosines of 0.610 and 0.366, respectively, signifying strong alignment with the second principal component.

The factor scores for the PCA are presented in Table 9. Factor scores in Principal Component Analysis (PCA) represent the projections of the original data on the principal components to summarize the information contained in the original data in a lower-dimensional space defined by the principal components [16]. Factor scores were computed by projecting the standardized data on the selected principal components. Each score is the dot product of the data vector and the corresponding eigenvector (principal component), as outlined in Goryainov & Goryainova [17]. The dots identified by the PCA were obtained from each tank in the experimental setup, representing triplicates of three application dosages of the rapid biofloc inoculum. The squared cosines in

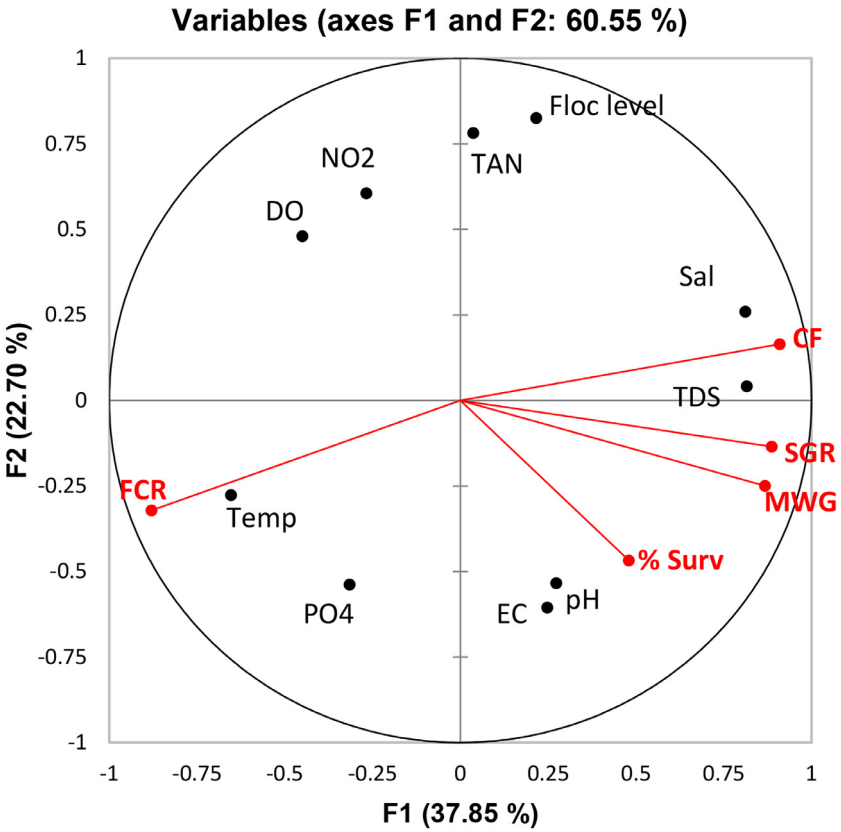

Fig. 2. Correlation cycle of the variables in the PCA of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Table 8
Squared cosines of the variables in the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Variables	F1	F2	F3	F4	F5	F6	F7	F8
Growth indices								
MWG (g)	0.752	0.062	0.158	0.006	0.012	0.002	0.001	0.006
CF	0.827	0.027	0.004	0.075	0.003	0.045	0.010	0.008
SGR ($g.day^{-1}$)	0.786	0.018	0.181	0.002	0.000	0.002	0.001	0.010
% Surv	0.230	0.219	0.006	0.096	0.331	0.008	0.109	0.000
FCR	0.775	0.103	0.046	0.001	0.002	0.072	0.000	0.001
Water quality								
TAN ($mg.l^{-1}$)	0.001	0.610	0.040	0.014	0.139	0.138	0.058	0.000
NO_2^- ($mg.l^{-1}$)	0.072	0.366	0.405	0.023	0.001	0.070	0.053	0.010
PO_4^{3-} ($mg.l^{-1}$)	0.099	0.290	0.062	0.493	0.052	0.002	0.001	0.000
Temp (°C)	0.426	0.077	0.421	0.021	0.034	0.001	0.017	0.004
DO ($mg.l^{-1}$)	0.202	0.230	0.016	0.200	0.240	0.096	0.015	0.000
EC ($\mu S.cm^{-1}$)	0.061	0.367	0.436	0.047	0.005	0.072	0.006	0.006
TDS ($mg.l^{-1}$)	0.665	0.002	0.042	0.270	0.012	0.002	0.006	0.000
Sal (ppm)	0.659	0.067	0.205	0.046	0.017	0.000	0.000	0.006
pH	0.075	0.286	0.558	0.067	0.009	0.000	0.003	0.002
Floc level ($ml.l^{-1}$)	0.047	0.681	0.043	0.111	0.073	0.029	0.014	0.001

Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

Table 8 further showed the strong associations between variables and their respective factors, further confirming MWG, CF, and SGR as key growth indicators under optimum water conditions. Furthermore, the factor scores (Table 9) illustrated the alignment of each observation with these factors, where 14 $ml.l^{-1}$ dosage was finally confirmed to possess balanced scores across growth and water quality indicators.

Table 9
Factor scores in the principal component analysis of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Points	Dosage (ml.l^{-1})	F1	F2	F3	F4	F5	F6	F7	F8
Obs1	7	−3.562	−1.291	2.400	−1.208	−0.684	−0.735	−0.285	−0.130
Obs2	7	−2.834	−0.785	−2.715	0.789	−0.858	0.724	−0.618	0.079
Obs3	7	−1.678	−1.516	−0.913	0.366	0.388	−0.175	1.344	0.061
Obs4	14	0.550	−0.416	2.110	0.887	0.944	0.340	−0.290	0.482
Obs5	14	3.649	−2.019	0.059	−1.808	-0.265	1.028	0.045	−0.103
Obs6	14	0.825	−0.640	−0.217	1.312	1.620	−0.285	−0.384	−0.414
Obs7	21	3.546	0.127	−0.949	0.491	−1.129	−1.392	−0.102	0.122
Obs8	21	−0.780	3.329	−1.342	−1.945	1.032	−0.217	−0.072	0.086
Obs9	21	0.283	3.210	1.567	1.117	−1.049	0.713	0.362	−0.183

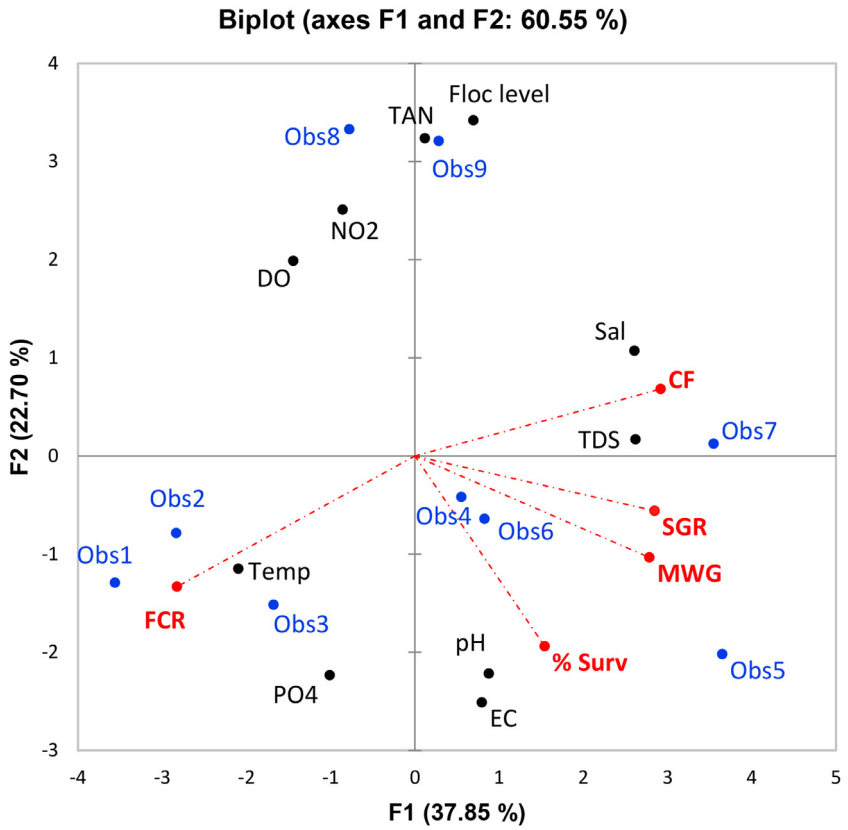

Fig. 3. Biplot of the PCA of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

A biplot (Fig. 3) was then constructed from the scores of data points and the loadings of variables in a Principal Component Analysis (PCA) for a single plot to visualize the relationships between the variables and data points in a lower-dimensional space defined by the principal components.

The percentage contributions of the observations in the PCA (Table 10) were computed using the relationship established in the biplot. High contributions indicated a strong association of the observations with the principal component and their significant contributions to the variance explained by that component.

The contributions of Obs1–3, which represents 7 ml.l^{-1} of the probiotic application dosage, had a mean of 6.83 in F1 and F2. In a similar vein, Obs 4–6 were observations points identified to represent 14 ml.l^{-1} which had a mean of 11.03 while Obs 7–9 represented 21 ml.l^{-1} and had a mean contribution of 9.30. Obs 4–6 were identified to account for the highest contribution to the relationships among the growth indices and water quality variables observed during the experiment. The mean calculations were limited to F1 and F2 because they account for 60.55 % of the variability in the measured variables.

The application of 1-way ANOVA did not reveal statistical variation across the different application dosages of probiotic in the culture system. However, the usage of Principal Component Analysis (PCA) provided information on the interactions among the variables, leading to the identification of 14 ml.l^{-1} as the most suitable probiotic dosage. This determination was based on observation

Table 10
Contribution of the observations (%) of the growth indices of *Litopenaeus vannamei* and water quality of the indoor biofloc aquaculture system.

Points	Dosage (ml.l^{-1})	F1	F2	F3	F4	F5	F6	F7	F8
Obs1	7	14.83	5.44	24.38	11.01	5.59	11.17	3.08	3.41
Obs2	7	5.71	2.01	31.21	4.69	8.78	10.82	14.41	1.26
Obs3	7	5.51	7.50	3.53	1.01	1.80	0.63	68.17	0.74
Mean (F1, F2)		*6.83*							
Obs4	14	13.59	0.57	18.84	5.93	10.64	2.39	3.17	46.75
Obs5	14	26.06	13.30	0.01	24.65	0.84	21.83	0.08	2.12
Obs6	14	11.33	1.34	0.20	12.97	31.30	1.68	5.57	34.50
Mean (F1, F2)		*11.03*							
Obs7	21	24.61	0.05	3.81	1.82	15.20	40.02	0.39	2.99
Obs8	21	1.19	16.17	7.62	28.52	12.72	0.98	0.20	1.49
Obs9	21	0.16	13.63	10.40	9.41	13.13	10.49	4.94	6.74
Mean (F1, F2)		*9.30*							

points 4, 5, and 6, which accounted for the highest variability, signifying the optimal probiotic dosage for achieving favorable growth performance of the shrimp and water quality conditions. While Tables 8 and 9 were intermediate steps to establishing the optimum probiotic dosage, the contribution of the observations (%) of the growth indices presented in Table 10 summarized the contribution of observations at each dosage level. Observations corresponding to the 14 ml.l^{-1} dosage (Obs4–6) had the highest mean contribution (11.03 %), particularly within F1 and F2, thereby marking it as the optimum level for a favorable balance between shrimp growth and water quality, as supported by the PCA.

Validation

The experiment was conducted under controlled indoor environmental conditions where replication of dosage levels was done to ensure result reliability. The validation of this research integrated Principal Component Analysis (PCA) and one-way ANOVA, which showed the multivariate relationships between probiotic dosages, growth indices, and water quality parameters. PCA effectively reduced dimensionality, aiding the identification of complex interaction patterns that were in agreements with the Pearson correlations. Furthermore, statistical significance for growth indices such as condition factor (CF) and food conversion ratio (FCR) observed in ANOVA and their high contribution in the principal component analysis confirms the consistency of findings across univariate and multivariate methods.

Limitations

This study was conducted in a controlled indoor biofloc system. This may limit its adoption in outdoor aquaculture environments where environmental variables are less stable. The findings are also constrained by the single-species focus on Litopenaeus vannamei, which potentially limits applicability to other aquaculture species with varying physiological responses to probiotics. Although the growth indices and selected water quality parameters were rigorously analysed, a detailed analysis of microbial communities and additional variables, such as immune response variables, could further enhance the understanding of the impact of probiotic dosage in biofloc systems.

Ethics statements

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Edward Terhemen Akange: Conceptualization, Methodology, Software. **Benjamin Orfega Kwaghvihi:** Data curation, Writing – original draft. **Olumide A. Odeyemi:** Writing – original draft, Visualization, Investigation, Writing – review & editing. **Hajar Rastegari:** Writing – original draft, Visualization, Investigation, Writing – review & editing. **Muhamad Shukri Shafie:** Data curation, Validation. **Ahmad Ideris Abdul Rahim:** Data curation, Validation. **Amyra Suryatie Kamaruzzan:** Data curation, Validation. **Siti Rozaimah Sheikh Abdullah:** Writing – review & editing, Funding acquisition. **Nor Azman Kasan:** Writing – original draft, Supervision, Writing – review & editing, Funding acquisition.

Data availability

Data will be made available on request.

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