

Optimization of the Pacific White Shrimp (*Litopenaeus vannamei*) Culture through Stocking Density, Probiotic, and Proportion of *Gracilaria verrucosa*

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ABSTRACT

This study aimed to determine the optimal conditions for cultivating the Pacific white shrimp (*Litopenaeus vannamei*) to achieve the desired outcomes, including an average daily growth (ADG) of 0.20-0.25g/day, a feed conversion ratio (FCR) between 1.5 and 2.0, a survival rate (SR) ranging from 75% to 100%, and a feed profit value after 120-150 days of shrimp cultivation. The research employed the Response Surface Methodology (RSM) using the Box-Behnken Design (BBD) with three key variables: stocking density, probiotic concentration, and the proportion of *Gracilaria verrucosa* powder. The findings revealed that the optimal conditions for *Litopenaeus vannamei* cultivation at 42 days shrimp age were achieved at a stocking density of 95 shrimp/m², a probiotic concentration of 2.34% (v/v), and a *Gracilaria verrucosa* powder proportion of 15% (w/w). These conditions resulted in an ADG of 0.122g/day, an SR of 95%, an FCR of 1.13, and a feed profit value of IDR 212.571, with a desirability score of 0.87 on 30 out of 31 generated solutions. The higher the age, the higher the FCR and ADG but the lower the SR. The study highlighted the potential of incorporating functional feed ingredients like *Gracilaria verrucosa*, this optimized approach to enhance growth performance, feed efficiency, survival, and profitability in *Litopenaeus vannamei* farming, paving the way for more sustainable industry practices. By integrating appropriate stocking densities with tailored probiotic supplementation and the inclusion of seaweed-derived feed ingredients, this research offers valuable insights into sustainable and economically viable shrimp aquaculture practices. The application of RSM in aquaculture optimization provides a robust framework for addressing complex interactions among variables, leading to precise recommendations for improving production efficiency and profitability.

INTRODUCTION

Stocking density, probiotic concentration, and the proportion of seaweed powder have been utilized as independent variables in optimizing the culture of *Litopenaeus vannamei* due to their positive correlations with key indicators of aquaculture success,

including average daily growth (ADG), feed conversion ratio (FCR), survival rate (SR), and feed profit value.

A strong positive correlation has been observed between stocking density and the inclusion of *Gracilaria corticata* with growth parameters of *L. vannamei*. Additionally, lower stocking densities, such as 70 shrimp/m², have been shown to yield higher harvest outputs (Kotiya & Vadher, 2021). Another study mentioned the condition of level setting factors (variables) to achieve maximum profit based on specifications is with a stocking density of 100 tails/m², feed protein content of 39.96%, and salinity of 20‰ (Hudi & Shahab, 2005). During the shrimp farming process indicators, SR, ADG, FCR and feed value, determine the success rate of shrimp farming. Shrimp rearing systems are dynamic with many processes affecting system performance. Shrimp production is influenced by stocking density, feeding rate and C:N ratio applied, future research should focus on the type of carbohydrate, feeding method and utilization of microbial communities can affect shrimp growth (Bossier *et al.* 2016). Stocking density determines the productivity of shrimp farming.

Probiotic concentration has also been highlighted as a crucial factor in improving the culture of *L. vannamei*. Higher inclusion levels of *Bacillus coagulans* at 1×10^8 CFU g⁻¹ in feed have been shown to enhance growth, gut morphology, gut microflora, immune responses, and resistance to *Vibrio parahaemolyticus* infections (Amoah *et al.*, 2019). Acute Hepatopancreatic Necrosis Disease (AHPND), a serious condition caused by *Vibrio* spp., significantly impacts the shrimp industry. Probiotics such as *Bacillus subtilis* AQAHBS001 have been found to enhance immune responses, gut integrity, and resistance against *Vibrio* infections, making them valuable in improving health and survival in intensive systems (Ferreira *et al.*, 2015; Kewcharoen & Srisapoome, 2019).

Shrimp feed is a very important concern, because almost 60% of the cost of cultivation is from this component. The use of red seaweed, such as *G. corticata*, has been reported to improve growth performance, survival rates, and total production of *L. vannamei* in non-water exchange systems (Fourooghifard *et al.*, 2017). The inclusion of *Gracilaria verrucosa* powder in shrimp feed has been recognized for its potential to enhance feed efficiency. Additionally, incorporating commercial feed with freeze-dried seaweed powders has shown positive effects on shrimp growth, energy status, antioxidant capacity, and ammonia-N tolerance (Shan *et al.*, 2019). Seaweed supplementation in shrimp feed has also been linked to improved gut microbiota and reduced mortality during viral outbreaks such as the White Spot Syndrome Virus (WSSV) (Schleder *et al.*, 2019). Furthermore, balanced diets including *Gracilaria lemaneiformis* have been shown to improve growth performance and stress resistance in *L. vannamei* (Yu *et al.*, 2016). Another research mentioned the use of 10% *Gracilaria arcuata* seaweed as fish feed showed an increase in weight, fish growth, and feed efficiency (Al-Asgah *et al.*, 2016). Commercial feed supplemented with freeze-dried *Ampithoe* sp. flour had positive effects on shrimp growth, energy status, antioxidants and ammonia-N tolerance (Anaya-Rosas

et al., 2019). Fresh macroalgae formulated with commercial feed in greenlip abalone culture as a carbohydrate source can save protein (Bansemer *et al.*, 2016). Long-term feeding of shrimp with diets containing 2.0g/ kg *Gracilaria tenuistipitata* induced expression of immune-related genes and showed enhanced immune response and resistance to *V. harveyi* (Arulkumar *et al.*, 2018).

To identify optimal conditions for *L. vannamei* cultivation, response surface methodology (RSM) has been applied. RSM estimates responses based on combinations of factor levels and determines optimal operating conditions to maximize system performance (Goren *et al.*, 2022). A significant advantage of RSM lies in its ability to generate extensive information from a limited number of experiments, minimizing time and cost. Its application has been widely reported in process optimization across various fields, including fermentation media, aquaculture processes, and enzyme production (Burkert *et al.*, 2004; Ruchi *et al.*, 2008). RSM has also been used for screening and optimizing antiviral compounds derived from marine plants and extracting phenolics and antioxidants using advanced methods (Chakraborty *et al.*, 2014; He *et al.*, 2018).

This study focuses on utilizing RSM to determine the optimal stocking density, probiotic concentration, and *G. verrucosa* powder proportion to enhance the growth performance, feed efficiency, survival rate, and profitability of *L. vannamei* farming systems.

MATERIALS AND METHODS

Experimental design and shrimp maintenance

The experiment was conducted over 42 days, during which whiteleg shrimp (*L. vannamei*) were cultivated under controlled conditions. Feed was administered three times daily at 07:00, 11:00, and 16:00 WIB. The feeding rate (FR) was adjusted according to the shrimp's body weight based on the Indonesian National Standard (SNI-01-7246-2006 (Kurniawan *et al.*, 2019), with FR percentages gradually decreasing from 15 to 5%. Water quality management was carried out by flushing and changing the water every 2 days by 10% of the total pool volume include vibrio colony count <10³ CFU, pH 7,5-8.5, alkalinity 100-250, and NH₃ <0.01.

Optimization factors

The optimization process was carried out by varying three independent factors: stocking density, probiotic concentration, and dietary composition. Stocking density levels of 80, 120, and 160 shrimp/m² were tested. Probiotics, comprising commercial bacterial and fungal strains, were administered at concentrations of 1, 2, and 3% v/v. The bacterial colony count was standardized at 10⁷ CFU/mL, and fungal concentrations were maintained at 10⁴ CFU/mL. Dietary variations included the incorporation of *G. verrucosa* powder at proportions of 0% (100% commercial feed), 15% (85% commercial feed), and 30% (70% commercial feed).

Performance measurements

At the conclusion of the experimental period, shrimp performance metrics were measured. Percentage weight gain (PG) was calculated using the formula:

$$PG(\%) = \frac{W_f - W_i}{W_i} \times 100$$

Where, W_f and W_i represent the final and initial shrimp weights, respectively (Xie *et al.*, 2019). The feed conversion ratio (FCR) was determined by dividing total feed consumption by the weight gain ($W_f - W_i$).

Survival rate (SR) was calculated as:

$$SR(\%) = \frac{N_f}{N_i} \times 100$$

Where, N_f and N_i are the final and initial shrimp counts, respectively.

The cost of feed was calculated by integrating the price of *G. verrucosa* powder into the commercial feed formula. This was achieved using the formula: Feed Cost = Commercial Feed Cost - [(Proportion of Commercial Feed × Feed Cost) + (Proportion of *G. verrucosa* × Seaweed Powder Cost)]. The resulting value represents the adjusted feed cost based on the varying proportions of *G. verrucosa* incorporated into the diet. Furthermore, the feed profit was determined by evaluating the relationship between feed cost, feed conversion ratio (FCR), and the market price of shrimp using the formula: Feed Profit = Feed Cost × FCR × Shrimp Price. This approach provided a comprehensive assessment of the economic feasibility of dietary modifications.

Experimental design using response surface methodology (RSM)

The response surface methodology (RSM) approach was employed to optimize shrimp performance outcomes. A Box-Behnken Design (BBD) was utilized, comprising 14 experimental runs, including two replicates at the central point. The design facilitated the assessment of interactions among the three factors while minimizing experimental costs and resources. A quadratic polynomial regression model was applied to predict responses, as represented by:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

Where, Y denotes the predicted response, β_0 is the intercept, β_i represents linear effects, β_{ii} indicates quadratic effects, and β_{ij} accounts for interaction effects. The regression coefficients were determined, and response surfaces were visualized using Design Expert 7.00 software (trial version, Stat-Ease Inc., Minneapolis, USA).

Statistical analysis

The impacts of stocking density, probiotic concentration, and dietary composition on survival rate, growth performance, FCR, and feed profitability were analyzed. Statistical validation of the model was performed through the analysis of variance (ANOVA). Optimization outcomes were predicted using RSM, enabling precise determination of the ideal conditions for sustainable *L. vannamei* aquaculture. Analysis of data was conducted using the Design Expert 7.00 application, including determining the desirability score.

RESULTS AND DISCUSSION

The discussion highlights the effects of stocking density, probiotic concentration, and *G. verrucosa* powder inclusion on the growth performance, feed efficiency, survival rate, and feed profitability of *L. vannamei*. The results provide insights into the interactions between these variables and their contributions to optimizing shrimp aquaculture systems (Table 1). Each parameter was analyzed to identify its role in achieving the most efficient and sustainable cultivation practices.

Table 1. Response optimization of ADG, SR, FCR of vaname shrimp farming process

Run Order	Stocking Density (shrimp/m ²) (X1)	Probiotic Concentration (%) (X2)	Proportion of <i>G. verrucosa</i> (%) (X3)	ADG (g/day)	SR (%)	FCR	Feed Profit (IDR)
1	80	3	15	0,12	0,98	1,20	128,05
2	80	2	30	0,13	0,96	1,13	182,52
3	120	1	30	0,12	0,93	1,26	212,57
4	160	2	0	0,11	0,80	1,60	133,96
5	80	2	0	0,12	0,94	1,23	120,90
6	120	1	0	0,14	0,50	2,01	54,51
7	160	3	15	0,08	0,90	1,96	74,45
8	120	2	15	0,12	0,91	1,29	168,73
9	120	2	15	0,11	0,92	1,39	160,91
10	80	1	15	0,1	0,95	1,48	84,22
11	120	3	0	0,1	0,90	1,57	91,72
12	160	2	30	0,11	0,70	1,83	137,04
13	160	1	15	0,13	0,50	2,17	74,92
14	120	3	30	0,11	0,90	1,43	177,75

Average growth performance (ADG)

The growth performance of *L. vannamei*, as measured by average daily growth (ADG) at 42 days of shrimp age, has been shown to vary significantly across different treatment conditions. Specifically, studies indicate that the ADG can range from 0.08 to 0.12g/ day, with optimal growth performance recorded at a stocking density of 95

shrimp/m², a probiotic concentration of 2.34% (v/v), and a dietary inclusion of 30% (w/w) *G. verrucosa*, achieving an ADG of approximately 0.12g/ day (**Won *et al.*, 2020; Kasan *et al.*, 2021**). This finding underscores the importance of both stocking density and probiotic supplementation in enhancing shrimp growth performance, suggesting a synergistic effect that warrants further investigation.

The interaction between stocking density and probiotic concentration is particularly noteworthy, as it has been associated with improved growth metrics in shrimp. Research by **Won *et al.* (2020)** highlights that specific probiotic strains can significantly enhance growth performance, immune response, and gut health in *L. vannamei*. Furthermore, the inclusion of probiotics has been linked to positive changes in gut morphology and immune responses, which are critical for the overall health and growth of shrimp (**Amoah *et al.*, 2019; Xie *et al.*, 2019**). This aligns with findings from **Amoah *et al.* (2019)**, who demonstrated that dietary supplementation with *Bacillus coagulans* not only improved growth performance but also enhanced intestinal morphology and immune responses in Pacific white shrimp.

Moreover, the use of red seaweed, such as *G. verrucosa*, as a dietary supplement has been shown to enhance growth and immune parameters in shrimp. **Fourooghifard *et al.* (2017)** reported that similar trends where the incorporation of red seaweed in shrimp diets resulted in improved growth performance and immune responses, indicating that such dietary inclusions can be beneficial for aquaculture practices (**Kasan *et al.*, 2021**). The positive effects of probiotics and dietary supplements on shrimp growth performance are further supported by evidence that highlights the role of gut microbiota in nutrient absorption and overall health, which is crucial for optimizing aquaculture productivity (**Wang *et al.*, 2019; Xie *et al.*, 2019**).

Feed efficiency (FCR)

The feed conversion ratio (FCR) is a critical metric in aquaculture, reflecting the efficiency with which feed is converted into body mass. In studies involving *L. vannamei*, the FCR has been reported to vary significantly, ranging from 1.13 to 2.17. The lowest observed FCR value of 1.54 was recorded under optimized conditions, indicating superior feed utilization (**MacLeod *et al.*, 2020**). This suggests that specific management practices can enhance feed efficiency, which is essential for sustainable aquaculture operations.

Stocking density has been identified as a significant factor influencing FCR. Higher stocking densities often lead to increased competition for resources, which can negatively impact feed conversion efficiency. This phenomenon is well-documented in aquaculture literature, where it has been shown that as stocking density increases, FCR tends to worsen due to stress and resource competition among shrimp (**El-Sayed, 2020; Rahman & Islam, 2021**). Therefore, managing stocking density is crucial for optimizing feed utilization and overall growth performance.

The inclusion of *G. verrucosa* in shrimp diets has been associated with improved feed efficiency, likely due to its rich nutrient profile and bioactive compounds that support digestion and overall health. Research indicates that the incorporation of seaweeds like *G. verrucosa* can enhance the nutritional value of diets, leading to better growth performance and feed conversion ratios in aquatic species (**Jitendrasinh, 2024**). For instance, studies have highlighted the benefits of using *G. verrucosa* in shrimp diets, noting its role in promoting better feed efficiency and growth metrics (**Wiyono, 2023**).

Moreover, the application of biofloc technology in shrimp farming has been shown to reduce FCR by enhancing the nutritional quality of the feed and improving water quality, which further supports shrimp health and growth (**El-Sayed, 2020; Raza, 2024**). The integration of probiotics and bioactive supplements into aquaculture feeds has also been linked to improved FCR, as these additives can enhance gut health and nutrient absorption, thereby leading to more efficient feed utilization (**Santoso & Sunadji, 2020; Han, 2024**).

In summary, optimizing feed conversion ratios in aquaculture, particularly for species like *L. vannamei*, involves careful management of stocking densities, strategic dietary inclusions such as *G. verrucosa*, and the potential benefits of biofloc systems and probiotics. These factors collectively contribute to enhanced feed efficiency, which is vital for the sustainability and profitability of aquaculture operations.

Survival rate (SR)

Survival rates (SR) in shrimp aquaculture can vary significantly, with reported rates ranging from 50 to 98%. An optimum survival rate of 95% has been achieved under specific conditions, namely a medium stocking density of 95 shrimp/m², a moderate probiotic concentration of 2.34%, and an inclusion of 30% *G. verrucosa* in the diet. The application of probiotics has been shown to significantly enhance survival rates, likely due to their role in improving immune function and resistance to pathogens. For instance, studies have demonstrated that the use of *Bacillus subtilis* as a probiotic can lead to improved immune responses and overall health in shrimp, which correlates with higher survival rates following pathogen exposure (**Widanarni et al., 2020; Won et al., 2020; Goh et al., 2022; Omar, 2024**).

Moreover, excessively high stocking densities have been linked to decreased survival rates, highlighting the critical balance required in aquaculture practices to mitigate stress-induced mortality. Research indicates that high stocking densities can lead to increased competition for resources and elevated stress levels among shrimp, which ultimately compromise their health and survival (**Ayuningrum et al., 2020; Knipe et al., 2020; Goh et al., 2022**). Therefore, maintaining optimal stocking densities is essential for maximizing survival rates and ensuring the sustainability of shrimp farming operations.

The integration of probiotics into shrimp aquaculture, coupled with careful management of stocking densities, is vital for enhancing survival rates and promoting the

health of shrimp populations. The evidence supports the notion that both the nutritional strategies employed and the environmental conditions-maintained play significant roles in the success of shrimp farming practices.

Feed profitability

The profitability of feed in shrimp aquaculture has been shown to vary significantly, with reported values ranging from IDR 54,508 to IDR 212,571. Under optimal conditions, the highest profit observed was IDR 212.571. This highlights the importance of feed formulation and the inclusion of cost-effective dietary supplements. Specifically, the significant contribution of *G. verrucosa* to feed profitability underscores its potential as a valuable dietary supplement in shrimp farming. The use of seaweed in aquaculture diets has been documented to enhance economic returns while simultaneously improving shrimp health and growth performance (Li *et al.*, 2020; Abbas *et al.*, 2023; Nauta, 2024).

Research indicates that dietary supplementation with seaweed, such as *G. verrucosa*, can lead to improved growth performance and immune responses in shrimp. For instance, studies have shown that polysaccharides derived from seaweed can regulate immune responses and can enhance intestinal morphology in shrimp, leading to better overall health and growth (Li *et al.*, 2020; Abbas *et al.*, 2023). Furthermore, the integration of seaweed into shrimp diets has been associated with increased profitability due to reduced feed conversion ratios and improved feed efficiency (Schleder *et al.*, 2018; Tsutsui *et al.*, 2020). This is particularly relevant in the context of rising feed costs, which have been a significant concern in the aquaculture industry (Arru *et al.*, 2019).

Moreover, the economic analysis of aquaculture feeds has highlighted the benefits of incorporating plant-based ingredients, including seaweeds, to optimize costs and improve profitability (Omeje *et al.*, 2023). The co-cultivation of shrimp with seaweed not only enhances shrimp growth but also contributes to better water quality management, which is crucial for sustainable aquaculture practices (Widowati *et al.*, 2021; Nauta, 2024). Thus, the strategic use of *G. verrucosa* and other seaweeds in shrimp diets represents a promising approach to enhance both the economic viability and sustainability of shrimp farming.

In conclusion, the integration of *G. verrucosa* as a dietary supplement in shrimp aquaculture not only improves profitability but also supports shrimp health and growth performance. The evidence from various studies underscores the multifaceted benefits of using seaweed in aquaculture, making it a valuable component in feed formulations.

Optimization and response surface analysis

The optimization process utilizing Response Surface Methodology (RSM) has indicated that the ideal conditions for cultivating *L. vannamei* involve a stocking density

effectiveness in optimizing various culture conditions. For instance, **Liu et al. (2020)**, demonstrated that the application of probiotics, such as *Bacillus subtilis*, significantly improved the survival and immune status of *L. vannamei* larvae, suggesting that the inclusion of probiotics can enhance the overall health and growth performance of shrimp. Similarly, **Vinatea et al. (2011)** explored the effects of environmental factors such as temperature and salinity on shrimp oxygen consumption, which can be critical in determining optimal stocking densities and feed formulations. This highlights the importance of understanding the physiological responses of shrimp to various environmental and dietary conditions.

Moreover, the findings from **Rezende et al. (2019)** support the notion that stocking density plays a crucial role in shrimp farming, as variations in water quality parameters were observed with different densities, which can affect shrimp growth and survival. This underscores the necessity of balancing stocking density with other factors such as feed composition and probiotic inclusion to achieve optimal growth conditions. The integration of seaweeds like *G. verrucosa* not only contributes to feed profitability but also enhances the nutritional profile of the diet, further supporting the health and growth of shrimp.

The results of vaname shrimp farming optimization through RSM - Box Bhenken Design (BBD) with Design-Expert 7.00 software obtained equation, significant level, lack of fit, Adj R-squared, and Adeq precision can be seen in Table (2).

Table 2. Mathematical equation of response of SR, ADG, FCR, and feed profit value

Parameter	Significant level	Lack of Fit	Adj R-squared	Pred R-squared	Adeq Precision
SR (Survival Rate) (P-value < 0.05 Significant)	Equation : $Y_1 = 0,842 - 0,116A + 0,1B + 0,0437C + 0.0925 AB - 0.0300AC - 0,108BC$ Significant (0.0129)	Not Significant	0.7194	0.3124	8.250
ADG (Average Daily Growth) (P-value < 0.05 Significant)	Equation : $Y_2 = 0,1143 - 0.0050A - 0,01B - 0,018AB - 0.0025AC + 0.0075BC$ Significant (0.0152)	Not Significant	0.7048	0.2087	9.5142
FCR (Feed Conversion Ratio) (P-value < 0.05 Significant)	Equation : $Y_3 = 1,54 + 0,315A - 0.0950B - 0.0950C$ Significant (0.0118)	Not Significant	0.7194	0.3124	8.2505
Feed profitability	Equation : $Y_4 = 175,45 - 11.79A + 5.09B + 37,85C - 10.82AB - 14.63AC - 19.51BC - 36.91A^2 - 47.88B^2 + 5.06C^2$ Not Significant (0.1861)	Not Significant	0.5246	-1.1782	4.8158

To get a prediction of the optimal solution in vaname shrimp farming is through a stocking density of 95 shrimp/m², probiotic concentration of 2.34% v/v and the proportion of *G. verrucosa* seaweed flour 30% b/w. The optimal solution, identified as solution 30 out of 31, yields the following results: SR value of 95%, ADG of 0.122g/hr, FCR of 1.22, and a feed profit of IDR 212,571, with a desirability score of 0.87. While the predicted value of the optimal response and the scale of importance of vaname shrimp culture can be seen in Table (3). The prediction of the optimal solution of aquaculture response can be seen in Fig. (1).

Table 3. Predicted value of optimal response and scale of importance for vaname shrimp farming

Solution 30 of 31 Response	Predicted Mean	Predicted Median	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop	Scale of Importance
ADG	0.122	0.122	0.008	0.005	0.109	0.134	0.074	0.169	*****
SR	0.952	0.952	0.085	0.053	0.826	1.078	0.456	1.449	***
FCR	1.222	1.222	0.224	0.113	0.969	1.476	0.096	2.349	***
Feed Profitability	212.571	212.571	33.829	23.882	146.263	278.878	-45.064	470.206	*****

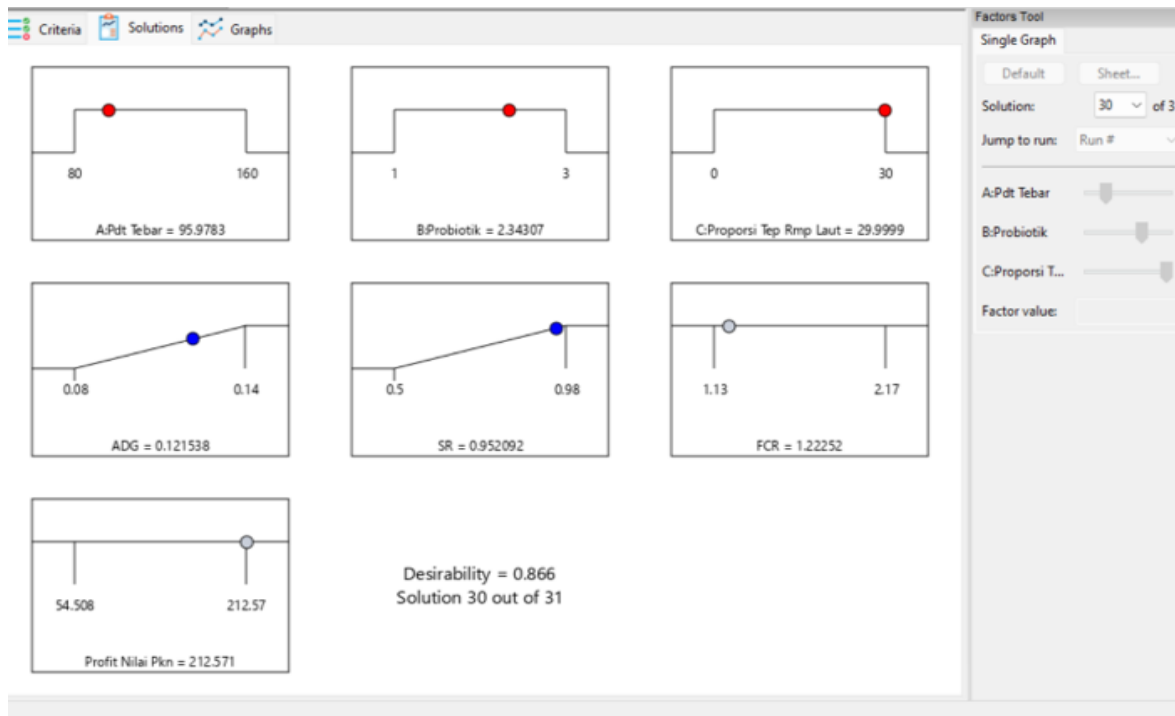


Fig. 1. Predicted optimal solution response to the vaname shrimp farming process

In conclusion, the optimization of shrimp farming conditions through RSM has provided valuable insights into the ideal parameters for cultivating *L. vannamei*. The interplay between stocking density, probiotic concentration, and dietary supplements like *G. verrucosa* is essential for maximizing growth performance and for ensuring the sustainability of shrimp aquaculture practices.

CONCLUSION

In this study, the optimal conditions for vaname shrimp farming, considering stocking density, probiotic concentration, and the proportion of *G. verrucosa* flour, were determined to be a stocking density of 95 shrimp/m², a probiotic concentration of 2.34% (v/v), and 30% (w/w) *G. verrucosa* flour. Under these conditions, the results included an ADG of 0.122g/hr, a survival rate (SR) of 95%, an FCR of 1.22, and a feed profit of IDR 212,571, with a desirability score of 0.87, as found in solution 30 out of 31. This study demonstrates the feasibility of optimizing *L. vannamei* culture through strategic adjustments in stocking density, probiotic concentration, and dietary supplementation with *G. verrucosa*. The findings provide a foundation for sustainable shrimp aquaculture practices, emphasizing the importance of integrating probiotics and functional feed ingredients to enhance productivity and economic viability. Proportion of *G. verrucosa* flour 30% (w/w) of commercial feed has the potential to be developed into a new industrial scale formula for vaname shrimp feed.

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