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Effect of Soybean Meal Substitution Using Raintree *(Samanea saman)* Seed Meal on the Physical Quality of Feed and Growth Performance of the Juvenile White Shrimp, *Litopenaeus vannamei*

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ABSTRACT

Efforts to find alternative raw materials to replace soybean meal in *Litopenaeus vannamei* feed are ongoing. Feed plays a critical role in promoting growth and survival and directly impacts production costs. The use of raintree seed meal as a protein source to replace soybean meal in *L. vannamei* feed has not yet been extensively studied. This study assessed the feasibility of using raintree seed meal fermented with a combination of different microorganisms as a replacement for soybean meal in feed, with the goal of improving the growth performance of *L. vannamei*. The research was conducted in two stages. First, Fourier transform infrared (FT-IR) analysis was performed to compare the functional groups of unfermented and fermented raintree seed meal. The fermentation process involved using a mixture of microbes at a concentration of 1.5 mL/100g and a fermentation time of 72 hours. Second, the study evaluated the replacement of soybean meal with fermented raintree seed meal at five different levels of substitution (0%, 25%, 50%, 75%, and 100%) in *L. vannamei* feed. The results showed an increase in intramolecular N-H amines and carboxylic acids and a decrease in O-H functional groups in the fermented raintree seed meal. The inclusion of 50% fermented raintree seed meal with mixed microbes significantly improved the feed's physical characteristics and shrimp growth performance, surpassing the effects observed in other treatments. The physical properties of the feed included a breaking speed of 64.73 minutes and a solids' dispersion of 10.86%. The growth performance metrics for *L. vannamei* included a feed consumption rate of 117.87g, feed efficiency of 81.44%, body glycogen content of 20.27%, absolute growth of 4.09g, and a survival rate of 89.16%. In conclusion, fermented raintree seed meal can effectively replace 50% of soybean meal in feed, enhancing the growth performance of *L. vannamei*.

INTRODUCTION

The availability of quality feed supplies is one of the supporting factors for the success of *Litopenaeus vannamei* cultivation, as it it influences both the growth rate **(Eid**

***et al.*, 2020)** and survival rate **(Suantika *et al.*, 2021)**, in addition to impacting manufacturing costs **(Emerenciano *et al.*, 2022)**. The feed can account for as much as 70% of the total production expenses in a financially successful aquaculture venture **(Yassien *et al.*, 2022; Muslmawy *et al.*, 2024)**. Moreover, the cost depends on the food's protein level, source, and type of ingredients derived from plant or animal sources. The Pacific white shrimp are considered carnivores requiring high protein feed ranging from 20 to 45% **(Lee & Lee, 2018; Elsayed *et al.*, 2022)**. Soybean meal is a raw material sourced from vegetable protein, and it is the most popular ingredient in shrimp and fish feed **(Wee *et al.*, 2023)**. The high competition for soybean meal—driven by its use in human food, livestock feed, and as a crucial ingredient in cosmetics and medicines—poses a significant threat to the future sustainability of the global soybean supply **(Robert & Tabor, 2009; Singh & Krishnaswam, 2022; Pope *et al.*, 2023; Majidian *et al.*, 2024; Nicholson *et al.*, 2024)**. Therefore, finding alternative protein sources to reduce dependence on soybean meal in *L. vannamei* feed production is essential. The search for alternative protein sources as a substitute for soybean meal in feed has been widely carried out. **Wang *et al.* (2020)** reported that supplementation of soybean seed flour using cottonseed meal as much as 87.44% with the addition of iron was able to improve the growth performance of the white shrimp. **Didyawati *et al.*, (2019)** postulated that the substitution of soybean meal using kapok seed oil dregs (Ceiba pandora) meal by 50% had a good effect on the level of feed consumption and growth rate of the white shrimp juveniles. **Han *et al.* (2022)** stated that 75% of soybean meal can be substituted with fermented cottonseed meal to increase the activity of the digestive enzymes of the shrimp. However, these results are not optimal to support the availability of a sustainable supply of the shrimp feed; thus, alternative feed is needed, one of which is raintree seed meal **(Aba & Hagan, 2013; Kasman *et al.*, 2022)**.

Mixed microbes fermented raintree seed meal is a source of high-quality feed **(Anwar *et al.*, 2023)**. Until now, its role in improving the physical properties of feed and the growth performance of the white shrimp has never been studied, therefore it was essential to research the substitution of soybean meal using raintree seed meal to support the growth performance of juvenile *L. vannamei*.

MATERIALS AND METHODS

Raintree seed meal fermentation was carried out from June to July 2022 in the Nutrition Laboratory of the Maros Brackish Water Cultivation Research Development Center, South Sulawesi Province, Indonesia, with chemical testing of raintree meal carried out in August 2022 in the integrated laboratory of the Bogor Agricultural Institute. The process of raising juvenile *L. vannamei* includes several stages:

1. **Preparation:** This involves acquiring juvenile *L. vannamei*, setting up the research containers (aquariums), and preparing feed ingredients and making the feed (December 2022 to January 2023).

2. **Rearing:** Juveniles *L. vannamei* are reared from February to January 2023.
3. **Chemical Testing:** Chemical testing of the feed was conducted in April 2023, followed by testing of the juvenile *L. vannamei* bodies in May 2023.

Research stages

Raintree seed samples and fermentation process: Raintree seeds were sun-dried until they reach 90% dryness, then crushed using a 60-mesh sieve. A mixture of microorganisms (*Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp.) was added at a dose of 4.5mL/ 100 grams to 100 grams of raintree seed meal. The mixture was fermented for 72 hours, as recommended by **Anwar *et al.* (2023)**. After fermentation, the raintree seed meal was steamed at 60°C for one minute to halt enzyme activity, cooled to room temperature, and then subjected to chemical testing in the laboratory.

Test feed preparation: The feed manufacturing process began with preparing raw materials, followed by mixing the feed ingredients, molding the feed, drying it, and finally packaging it. Feed formulation involves substituting soybean meal with cashew seed meal fermented with mixed microbes. This follows the approach of **Zainuddin *et al.* (2014)**, with modifications to replace soy meal with raintree seed meal, as detailed in Table (1)

Table 1. Research feed formulation

Feed raw material	Feed A	Feed B	Feed C	Feed D	Feed E
Fish meal	29	28	26	23	22
Shrimp head meal	9	9	9	9	9
Soy meal	27	20,25	13,75	6,75	0
Fermented raintree seed meal	0	6,75	13,75	20,25	27
Corn meal	18	18	18	18	18
Bran meal	10	10	10	10	10
Flour	3	4	6	9	10
CMC	2	2	2	2	2
Vitamin mixed	2	2	2	2	2
Total	100	100	100	100	100
Proteins (%)**	33,21	34,12	35,43	34,49	34,47
Crude fiber (%)**	5,70	5,42	4,12	4.08	4.03
NFE (%) **	33,91	33,91	33,91	33,91	33,91
Fat (%) **	14,31	14,31	14,31	14,31	14,31
Energy (kkal/g)*	4858	4858	4858	4858	4858

Note: *It is calculated based on digestible energy according to **Watanabe (1988)**, which states that one gram of protein contains 5.6 kcal/g; one gram of carbohydrates contains 4.1 kcal/g, and one gram of fat contains 9.4 kcal/g; ** Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University.

Research treatment and design

Maintenance was designed in a completely randomized design (CRD), with five treatments each, repeated three times. The feed formulation was substituted for soybean meal with raintree seed meal, which was fermented using mixed microbes. The order of treatment was as follows:

Treatment A: 100% soy meal without fermentation.
Treatment B: 25% fermented raintree seed meal and 75% soybean meal.
Treatment C: 50% fermented raintree seed meal and 50% soybean meal.
Treatment D: 75% fermented raintree seed meal and 25% soybean meal.
Treatment E: 100% fermented raintree seed meal.

Preparation of test feed

Post larval stage (PL) 30 *L.vannamei* were obtained from the Punaga Takalar pond, Installation of the Brackish Water Aquaculture Fisheries Research Institute, Maros. The rearing of 600 test animals began with an acclimatization process to the environment, such as temperature, salinity of the rearing media, and acclimatization to the test/control feed for 6 days; then, the initial weight was weighed (0.08± 0.00g). For 60 days (until harvest), treatment feed was given 4 times a day at 06:00, 12:00, 18:00, and 22:00 WITA with a feeding percentage of 10% of the test animal biomass with a protein content of 35%. Sampling was conducted every 10 days to determine the increase in weight of the test animals and to adjust the amount of feed given. Water changes were carried out for every sampling, as much as 30%. Juvenile *L. vannamei* stage post larvae (PL) 30, sourced from hatcheries in the Takalar Regency community, were adapted to feed control for four days in holding tanks. Then, the shrimp were sorted with an average initial weight of 0.08g and stocked with a dense stocking of 40 fish/40 liters **(Supono *et al.*, 2022)**, with a salinity of 25ppt, in 15 glass aquariums measuring 50 x 30 x 35cm3 and equipped with aeration installations and heaters. All containers were closed using black warding. Feeding was continued with a frequency of four times (06.00, 12.00, 18.00, and 21.00 WITA) a day as much as 10% of the shrimp's body weight following the modified method of **Zainuddin and Haryati (2014)**.

During the rearing process, the water quality was in optimum conditions to support the growth of juvenile *L. vannamei*. The water was changed using the siphon method every day, namely in the morning and evening before feeding. To avoid cannibalism, each aquarium was given shelter as a piece of paragon pipe, measuring 32mm in diameter with a length of 15cm.

Parameters

Pellet breakdown speed

The breakdown speed of pellets was assessed by measuring the time (in minutes) required for them to soften or disintegrate in water. To conduct the measurement, five identical pellets were placed in a beaker containing one liter of seawater with a salinity of 20 ppt. The pellets were gently pressed with the index finger every 15 minutes to check for softness. Observations continued until the feed was damaged or completely disintegrated **(Saade *et al.*, 2011)** ... (1)

Pellet solid dispersion

Solid dispersion was tested using a modified method **(Balazs, 1973)**. The dry weight was determined by converting it to the water content and calculated using the following procedure: Five grams of pellets were placed into a gauze box measuring 10x10 cm^2 with pores of approximately 1mm in size. The initial weight of the gauze box was recorded. The box was then submerged in water with a salinity of 20ppt in an aquarium equipped with an aeration system at room temperature. After four hours, the pellets remaining in the gauze box were dried in an oven at 105°C for 10 hours, then cooled in a desiccator and weighed until a constant weight was achieved. The dry weight was then calculated by converting it to the water content.

$$Dispersion\ of\ solids = \frac{dry\ weight\ of\ the\ final\ feed}{the\ dry\ weight\ of\ the\ initial\ feed} \text{ x } 100\% \ldots (2)$$

Total feed consumption (TFC)

Total feed consumption was calculated following the equation of **Weatherley (1972)**:

(TFC) TFC (g) = 1^{st} day of feed (g) + 2^{nd} day of feed (g) + .. + n^{th} day of feed (g) … (3)

***Efficiency feed utilization* (EFU)**

Efficiency feed utilization was calculated according to the equation reported in the study of **Tacon (1987)**:

$$\text{EFU (\%)} = \frac{\text{final weight} - \text{initial weight}}{\text{Weight of diet konsumed}} \text{ x } 100 \ \ldots (4)$$

The glycogen content body Litopenaeus vannamei

The glycogen content body was calculated following the equation of **Carrol *et al.* (1995)**:

$$\text{glycogen } (\frac{\text{mg}}{\text{g}} \text{ sample}) = \frac{\text{abs.}\frac{\text{spl}}{\text{abs}}\text{.std x kons.std x Df x } 1/1000}{\text{Sample weight(g)}} \ \ldots (5)$$

Abs. spl = sample absorbance at λ 670nm; Abs.stda = absorbance standard; Kons. Std = Standard concentration (500µg/ mL); Df = dilution factor (5X), and 1/1000 = change from micrograms to milligrams.

Absolute growth

Absolute growth was calculated following the equation of **De Silva and Anderson (1995)**:

Absolute growth (A.G) (g) = Final body weight (g) - Initial body weight (g) … (6)

Survival rate

Survival rate by **Effendie (1997)**:

$$\text{Survival rate (S.R) (\%)} = 100 \text{ x } \frac{\text{final count}}{\text{Initial count}} \ldots (7)$$

Fourier transform infrared (FTIR) analysis

Sample analysis using FTIR (Fourier transform infrared) can identify functional groups in the sample. 2mg of raintree seed powder sample was mixed with 200mg of Kalium Bromide, homogenized, and then formed into pellets using a hydraulic pump to form thin flakes. The sample spectrum was measured using F (S spectrum one FT-IR Spectrometer C69526, Perkins Eimer precisely, connected to a PC equipped with OPUS software) in the IR area (400-4000cm^{-1}). The spectrum data obtained from the absorption points were then converted into point table data format as a correction baseline for preliminary purposes. The absorption value was adjusted so that the highest absorption value is one, and the lowest absorption value is zero.

Proximate analysis

The sample's protein, fat, ash, carbohydrate, fiber, and water content were determined using a proximate analysis method specified by the Association of Official Analytical Chemists **(AOAC, 2005)**. The Kjeldahl technique was employed to assess the protein level, while the Soxhlet method was utilized to evaluate the fat content. The water and ash contents were assessed using gravimetric methods. Moreover, the carbohydrate content was estimated manually using the results of the approximation analysis.

Water quality

Water quality testing was carried out in the water quality laboratory of the Faculty of Marine and Fisheries Sciences, Hasanuddin University. A water quality checker was employed to assess the parameters of temperature, pH, salinity, and dissolved oxygen (DO). The variables of temperature, salinity, and pH were evaluated bi-daily. At the same time, oxygen levels were examined once a day, and ammonia levels were monitored every week.

Data analysis

The results of feed chemical tests were analyzed descriptively by comparing the results obtained between treatments and supporting literature. Meanwhile, the results of physical examinations, including breaking speed and solid dispersion, and feed biological tests, which include feed consumption level, feed utilization efficiency, shrimp body glycogen content, absolute growth, and survival of juvenile *L.vannamei* shrimp in each treatment, were analyzed using variance if any. Differences between treatments were then continued using the Duncan test at a 95% confidence interval using the SPSS version 26 program. Water quality data were evaluated descriptively.

RESULTS

The spectrum results of FTIR analysis of raintree seed meal without fermentation and those fermented with mixed microbes have very significant differences in the IR spectrum. It can be seen from the functional groups that there is a shift in the peak, the high and low peak intensity, and the width and narrowness of the wave number, which is indicated by the addition of mixed microbes. The FTIR spectrum results show that raintree seed meal experiences a wave number change characterized by forming new bonds. It can be seen from the IR spectrum that raintree seed meal fermented by optimum mixed microbes experiences a change from the O-H group to the N-H group. The formation of N-H groups indicates an increased protein content caused by bond deformation in the protein **(Ajayi *et al.*, 2019)** of raintree seed meal. The N-H group in the formed fermented raintree seed flour proves that cross-linking occur between the concentration of amino acids and protease enzymes in the raintree seed meal. It is also evident from the visible sharpening or shift in wave numbers in the C-H, C=C, C-O, and C-X functional groups, which experience strengthening of the functional groups. In addition, research shows that the strengthening of the infrared spectrum peak is characterized by a decrease in transmittance or sharpening of the valley, which indicates a strengthening of functional group bonds, indicating that several functional groups are bound in a material **(Syah *et al.*, 2022)**.

The results of functional group analysis or Fourier transformed infrared spectroscopy (FTIR), identified in Fig. (4A and B), show that O-H stretching, C-H stretching, C-H bending, and C-O stretching occur. The wave numbers for each functional group are different between images A and B, indicating changes in the beans' chemical composition after fermentation.

Raintree seed meal in this study showed a slightly higher O-H stretching wave number after fermentation (3289/cm) than before fermentation (3283/cm). The wave numbers for C-H bending are very close, with slight differences, but remain in the same range before and after fermentation (2881/cm). The C-O stretching wave number was 1048/cm before fermentation and 1047/cm after fermentation using a mix of microorganisms.

The N-H bending wave number was 1527/cm before fermentation and 1539/cm after fermentation, which indicated a slight increase in the functional groups of raintree seed meal after fermentation. Moreover, the N-H bending wave number was 1238/cm before fermentation and 1293/cm after fermentation, indicating a significant increase in this functional group after fermentation. Furthermore, the C=O stretching wave number was 1651/cm before fermentation and 1652/cm after fermentation, which shows a slight increase in functional groups in raintree seed meal using the mixed microbes, as shown in Fig. (1).

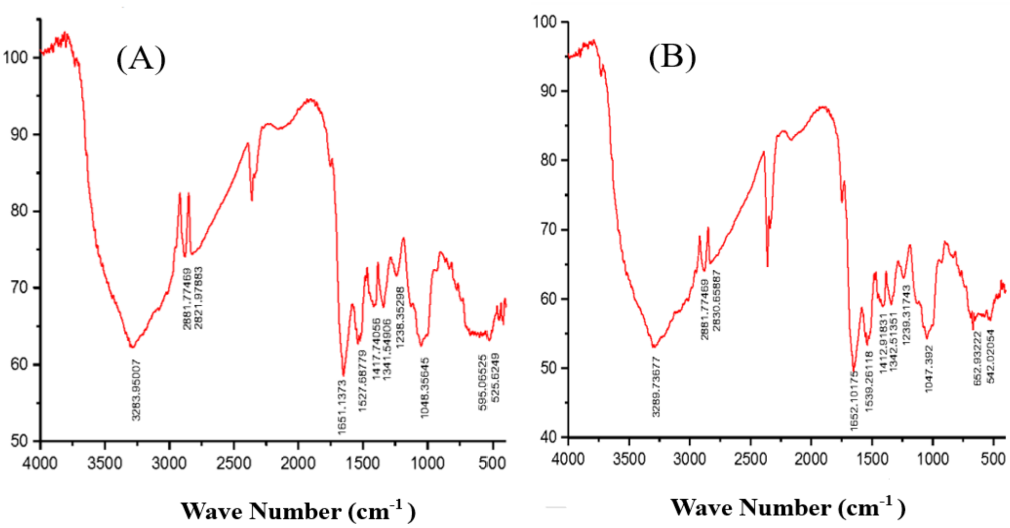

Fig. 1. Functional group analysis /PTIR raintree seed meal: **(A)** Without fermentation; **(B)** Fermented using mixed microbes

Table 2. Value of feed breaking speed (minutes), feed solids dispersion (%), total feed consumption (TFC), feed utilization efficiency (FUE), body glycogen content (%), absolute growth and survival (SR) during the study

Parameter measured	Feed substitution %				
	A (0 %)	B (25%)	C (50%)	D (75%)	E (100%)
Pellet breaking speed (minutes)	50.59±0.07^{a}	53.84±0.07^{b}	64.73±0.26^{c}	65.39±0.58^{c}	65.46±0.50^{c}
Dispersion of solids (%)	15.60±0.52^{c}	12.66±0.30^{b}	10.86±0.61^{a}	10.80±0.52^{a}	10.26±0.30^{a}
Total feed consumption (g)	82.95±1.44^{a}	87.75±1.35^{a}	117.87±6.02^{d}	103.53±0.85^{c}	93.58±1.09^{b}
Feed utilization efficiency (%)	70.45±0.09^{a}	74.17±0.33^{b}	81.44±2.18^{d}	77.70±1.10^{c}	75.04±1.10^{b}
Body glycogen content (%)	13.43 ± 0.02^{a}	15.65 ± 0.18^{b}	20.27 ± 0.09^{e}	19.15 ± 0.16^{d}	18.24 ± 0.06^{c}

Absolute growth (g)	2.93±0.00[a]	3.09±0.04[b]	4.09±0.03[e]	3.82±0.01[d]	3.28±0.01[c]
Survival rate (%)	73.33 ±1.43[a]	76.66 ±1.44[b]	89.16 ±1.44[e]	84.16 ±1.43[d]	80.33 ±1.44[c]

Table (2) shows that treatment C's best results were obtained (50% raintree seed meal).

Feed breaking speed (F.B.S)

The result of the graph of the orthogonal polynomial test is presented in Fig. (2).

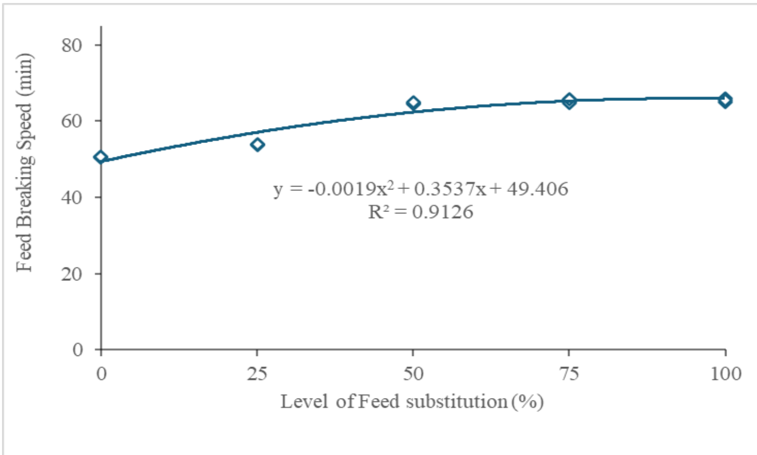

Fig. 2. Relationship between raintree seed fermentation substitution in feed with F.B.S

Based on the orthogonal polynomial relationship test with a quadratic pattern ($y = -0.0019x^2 + 0.3537x + 49.406$) and $R^2 = 0.9126$ with the optimum point in treatment C (50% substitution of fermented rain tree seed meal in artificial feed), the dose obtained from the equation (93.07%) is capable of producing a feed breaking speed of 65.86 minutes. The R^2 value indicates that 91.26% of the variation in feed breaking speed is explained by the substitution of raintree seed meal, while other unknown factors account for 8.74%.

Solid dispersion (S.D.)

The result of the graph of the Orthogonal Polynomial test is presented in Fig. (3).

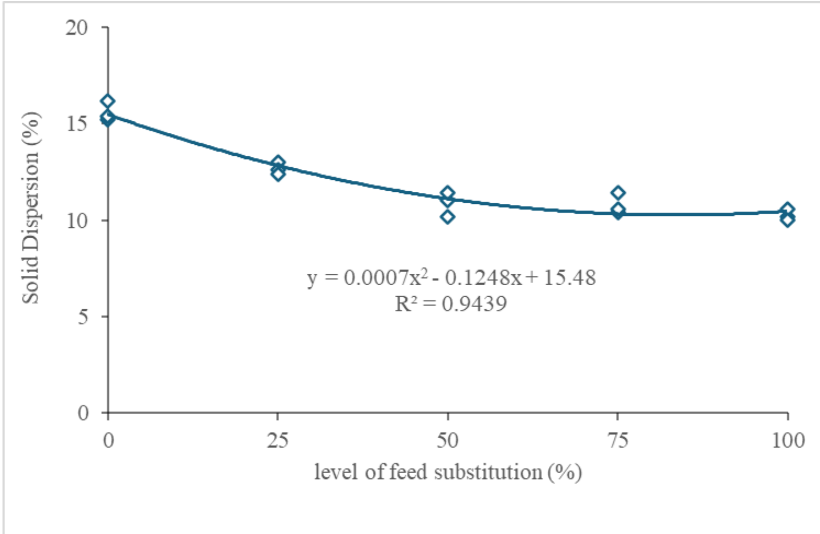

Fig. 3. Relationship between rain tree seed fermentation substitution in feed with S.D

Based on the orthogonal polynomial relationship test with a quadratic pattern ($y = 0.0007x^2 – 0.1248x + 15.48$) and R^2 value of 0.9439, the optimum feed solid dispersion (SD) is achieved with treatment C, which involves 50% substitution of fermented raintree seed meal in artificial feed. According to the equation, this substitution level results in a feed solid dispersion of 15.47%. The R^2 value shows that 94.39% of the variation in solid dispersion is due to the substitution of raintree seed meal, while other unknown factors account for 5.61%.

Total feed consumption (T.F.C.)

The results of the orthogonal polynomial test are shown in Fig. (4). Based on the quadratic relationship ($y = -0.0085x^2 + 0.9935x + 79.167$) with an R^2 value of 0.6592, the optimum feed consumption (T.F.C.) is achieved with treatment C, which involves a 50% substitution of fermented raintree seed meal in artificial feed. According to the equation, this substitution level results in a total feed consumption of 84.77%. The R^2 value indicates that 65.92% of the variation in total feed consumption is due to the substitution of raintree seed meal, while other unknown factors account for 34.08%.

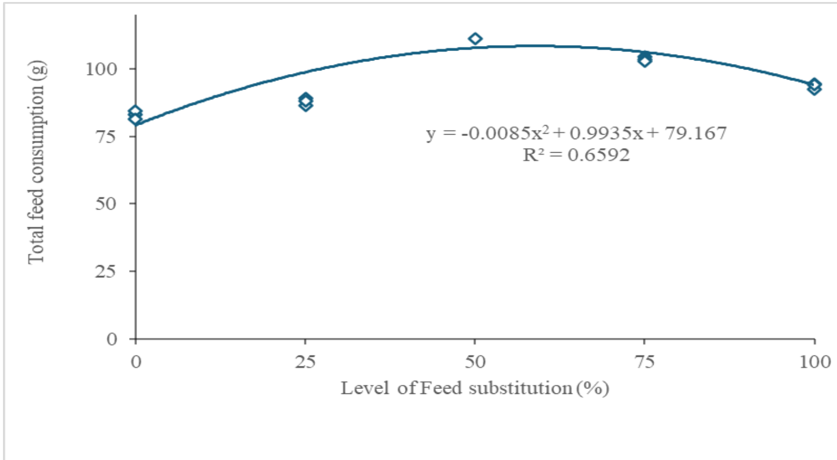

Fig. 4. Relationship between rain tree seed fermentation substitution in feed with T.F.C. of *L.vannamei*

Feed utilization efficiency (F.U.E.)

The result of the graph of the Orthogonal Polynomial test is presented in Fig. (5).

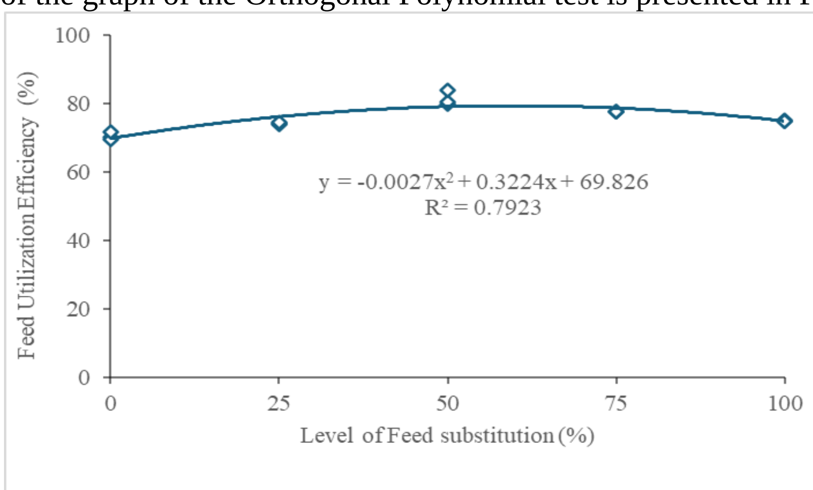

Fig. 5. Relationship between raintree seed fermentation substitution in feed with F.U.E. of *L.vannamei*.

Based on the orthogonal polynomial relationship test with a quadratic pattern ($y = -0.0027x^2 + 0.3224x + 69.826$) and an R^2 value of 0.7923, the optimum feed utilization efficiency (F.U.E.) is achieved with treatment C, which involves a 50% substitution of fermented raintree seed meal in artificial feed. According to the equation, a substitution level of 23.02% results in a feed utilization efficiency of 75.81%. The R^2 value indicates that 79.23% of the variation in feed utilization efficiency is due to the substitution of raintree seed meal, while 20.77% is attributable to other unknown factors.

Body glycogen content (B.G.C.) of juvenile *L. vannamei*

The result of the graph of the orthogonal polynomial test is presented in Fig. (6).

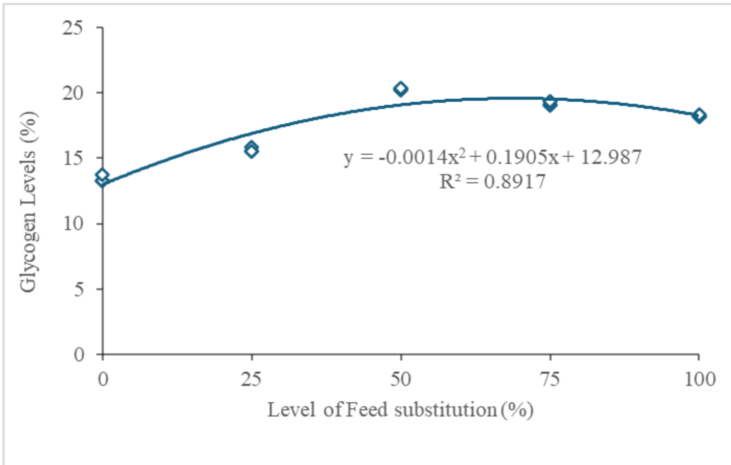

Fig. 6. Relationship between rain tree seed fermentation substitution in feed with B.G.L. of *L.vannamei*

Based on the Orthogonal Polynomial relationship test with a quadratic pattern ($y = -0.0014x^2 + 0.1905x + 12.987$) and an R^2 value of 0.8917, the optimum body glycogen content is achieved with treatment C, which involves a 50% substitution of fermented raintree seed meal in artificial feed. According to the equation, a substitution level of 68.03% results in a body glycogen content of 19.46%. The R^2 value indicates that 89.17% of the variation in glycogen levels is due to the substitution of raintree seed meal, while 10.83% is attributable to other unknown factors.

Absolute growth (A.B.)

The result of the graph of the Orthogonal Polynomial test is presented in Fig. (7).

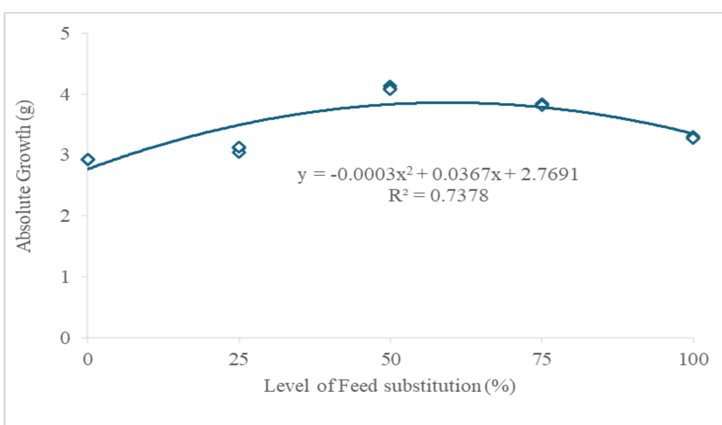

Fig. 7. Relationship between raintree seed fermentation substitution in feed with A.B. *L.vannamei*

Based on the Orthogonal Polynomial relationship test with a quadratic pattern ($y = -0.0003x^2 + 0.0367x + 2.7691$) and an R^2 value of 0.7378, the optimum absolute growth is achieved with treatment C, which involves a 50% substitution of fermented raintree seed meal in artificial feed. According to the equation, a substitution level of 61.16% results in an absolute growth of 3.79 grams. The R^2 value indicates that 73.78% of the variation in absolute growth is due to the substitution of raintree seed meal, while 26.22% is attributable to other unknown factors.

Survival rate (S.R.)

The result of the graph of the orthogonal polynomial test is presented in Fig. (8).

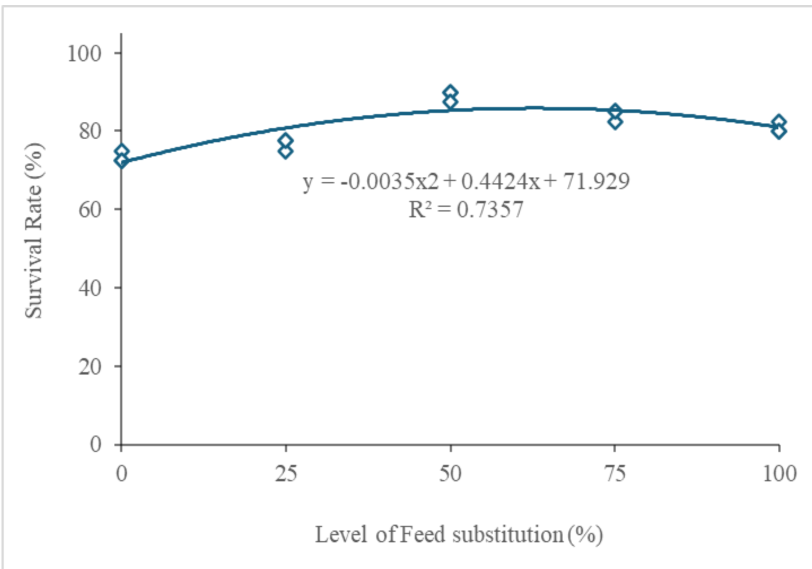

Fig. 8. Relationship between rain tree seed fermentation substitution in feed with S.R. of *L.vannamei*

Based on the orthogonal polynomial relationship test with a quadratic pattern ($y = -0.0035x^2 + 0.4424x + 71.929$) and an R^2 value of 0.7357, the optimum substitution level is achieved with treatment C, involving a 50% substitution of fermented raintree seed meal in artificial feed. According to the equation, a substitution level of 63.2% results in a survival rate of 85.27%. The R^2 value indicates that 73.57% of the variation in survival rate can be attributed to the substitution of raintree seed meal, while 26.43% is due to other unknown factors.

Water quality parameters are crucial for supporting the white shrimp cultivation **(Heriansah *et al.*, 2022)**. During the research, the water quality parameters, including temperature, pH, and salinity, remained relatively stable, as the research was conducted in a controlled environment with proper monitoring. Additionally, the parameters of dissolved oxygen, ammonia, and total suspended solids (TSS) (Table 3) were found to be suitable for the growth of juvenile *L. vannamei*.

Tabel 3. Results of air quality measurements in all treatments

Variable	Treatments					References
	A	B	C	D	E	
Temperature	28	28	28	28	28	>27^0C (SNI,2016)
Salinity	27	27	27	27	27	26-32 (SNI,2016)
pH	7,5-7,7	7,5-7,9	7,5-7,8	7,7-7,8	7,6-7,8	7,5-8,5 (SNI,2016)
Dissolved oxygen (ppm)	4,48-8,32	6,08-8,32	5,12-6,08	5,126,72	5,76-6,08	3-8 (SNI, 2016)
TSS (NTU)	101-132	118-224	110-202	135-214	147-226	< 400 (SNI, 2016)
Ammonia (ppm)	0,005-0,010	0,010-0,090	0,040-0,080	0,038-0,090	0,030-0,087	<0,1(SNI8037.1:2014)

DISCUSSION

Fourier transform infrared (FTIR) analysis

The public has used fermentation by utilizing the performance of bacterial microorganisms, protozoa, fungi/molds, and yeast as a traditional food processing technique with simple and cheap methods **(Jannathulla *et al.*, 2017; Jassim *et al.*, 2024)**. Several researchers have reported the use of microorganisms as fermentation agents **(Helmiati *et al.*, 2020; Abbas *et al.*, 2022)**. They were utilized to increase nutritional value **(Córdova-Murueta *et al.*, 2017)** as well as to reduce anti-nutritional substances in fish and shrimp feed raw materials **(Jannathulla *et al.*, 2017)**. Fermentation by combining microorganisms of *Bacillus* sp., *Saccharomyces cerevisiae*, and *Rhizopus* sp. has proven potential to increase the nutritional value and to reduce the anti-nutritional substances of seaweed flour as a raw material for fish and crab feed compared to using a single microorganism **(Aslamyah *et al.*, 2017)**. The same thing was done by **Zhang *et al.* (2021)** through combining *Lactobacillus plantarum* and *Saccharomyces cerevisiae* to improve the quality of commercial feed by fermenting it, and it has been proven to increase digestive enzyme activity and the growth of *L. vannamei*. Moreover, **Vidriales *et al.* (2021)** stated that fermenting rice bran using *Bacillus* sp. and *Lysinibacillus* resulted in an increase in the enzyme activity and the growth of *L. vannamei*.

Anwar *et al.* (2023) demonstrated that thefermentation of raintree seed meal using a mixture of *Bacillus* sp., *Saccharomyces cerevisiae*, and *Rhizopus* sp. microorganisms with a dose of 4.5mL /grams and a fermentation time of 72 hours, proved to reduce crude fiber and tannin and to increase soluble protein, which is efficiently utilized by the organism. The results of functional group analysis in this study also showed differences in the chemical composition of raintree seed meal before and after fermentation. Changes in chemical composition are indicated by an increase in intramolecular N-H amines and carboxylic acids and a decrease in O-H functional groups. The raintree seed meal in this study showed a slightly higher O-H stretching wave number after fermentation (3289/cm) than before fermentation (3283/cm). The wave numbers for C-H bending are very close, with slight differences, though remaining in the same range before and after fermentation (2881/cm).

The C-O stretching wave number was 1048/cm before fermentation and 1047/cm after fermentation using mixed microbes. The N-H bending wave number was 1527/cm before fermentation and 1539/cm after fermentation, which indicated a slight increase in the functional groups of raintree seed meal after fermentation. The N-H bending wavenumber was 1238/cm before fermentation and 1293/cm after fermentation, indicating a significant increase in this functional group after fermentation. The C=O stretching wave number was 1651/cm before fermentation and 1652/cm after fermentation, which shows a slight increase in functional groups in raintree seed meal after fermentation using a mix of microorganisms. The research results were also strengthened by an increased organic matter digestibility with *in vitro* dry matter digestibility as an initial indication that raintree seed flour is fermented, easy to digest, and contains quality nutrients. Based on the results of this research, it was concluded that fermented trembles seed meal mixed microbes are suitable for use as raw material for *L. vannamei* feed to reduce the use of soybean meal as a source of vegetable protein.

Pellet breaking speed and dispersion of pellet solids

This study shows that the rate at which pellet solids break down and disperse highlights the contrast between feed containing fermented cashew seed flour as a substitute and feed without any substitution (control). Feed substituted with fermented raintree seed meal showed a better breaking speed and solid dispersion than the control treatment feed (Table 2). This is caused by fermented raintree seed meal, which is mixed with feed raw materials resulting in a soft texture, forming a more compact bond adhering to each other. In contrast, the texture of unfermented soybean meal in the control treatment is stiffer/coarse and hollow. Fermented raw materials produce a finer texture, resulting in denser feed solids. Researchers reported that fermented feed raw materials produce a finer texture, resulting in denser feed solids **(Bogevik *et al*., 2021)**. This fact, proven by functional group analysis, shows changes in the chemical structure of raintree seed flour before and after fermentation using mixed microbes (Fig.1A, B). The change in chemical structure during fermentation is thought to be due to the structure of the cellulose molecule, which consists of 1,4 beta bonds and D-glucose with an elongated and rigid structure, which is hydrolyzed to become glucose ($C_6H_{12}O_6$), which contains simpler compounds. This finding is in line with the results of **Kurniawan *et al.* (2019)**, who stated that fermented moringa leaf flour has a more compact texture and structure, thus it has a better feed stability in water compared to unfermented moringa leaves as the gourami fish feed (*Osphronemus gouramy*). The results of this research are higher than those of **Saade *et al.* (2011)**. The breaking speed and solid dispersion of pellets made from *G. gigas* seaweed flour adhesive are 55.00 minutes and 10.86% **(Puteri *et al.*, 2021)**. Pellet breaking speed was 34.23 minutes on fish feed added with chicken manure. Fermented soybean meal *Monascus purpureus* M-32 as feed for the Pacific white shrimp *L. vannamei* can improve growth performance, immunity parameters, disease resistance, gut morphology, gut microbiota, and metabolism of the white shrimp **(Wang *et al.*, 2024)**. Given the results of this research, feed quality is greatly influenced by several factors, such as the type of raw material, nutritional composition, and disintegration in water **(Jannathulla *et al.*, 2019; Cahya *et al.*, 2022)**. Pellet breaking speed and solid dispersion are essential in making feed since feed tends to break down quickly and easily and decompose in water, even being not completely consumed by fish. As a result, the feed given could be more effective and efficient.

Growth performance

Growth performance is all factors or parameters that are directly related to or those influencing the increase in number, size, and dimensions at the cell, organ, and body tissue level of an individual/organism. According to **Li *et al*. (2022)** and **Novriadi *et al*. (2023)**, good feed quality is an essential factor influencing the growth performance of the Pacific white shrimp. The research showed that the highest total feed consumption for the juvenile *L. vannamei* was at a substitution level of 50% soybean meal with fermented raintree seed meal. This is thought to be significantly influenced by the palatability of the feed **(Nunes *et al.*, 2022)**. This is closely related to the high palatability of the feed **(Haetami *et al.*, 2017)**. It was proven that the response of the white shrimp *L. vannamei* when fed was very active in all feed treatments substituted for fermented raintree seed meal compared to the control treatment. The results showed that fermented raintree seed meal in the feed had higher feed palatability than the control treatment. This condition is caused by the level of substitution of raintree seed meal fermented by different microbes in the feed, resulting in changes in the feed's palatability, color, taste, and flavor. **Yuan *et al.* (2021)** reported that feed content and physical properties, such as size, shape, color, texture, taste, and flavor, influence total feed consumption.

In contrast to the treatment at 0% substitution level (control), the lowest average value of feed consumption level was obtained. This is influenced by the stability of the feed in water (solids dispersion and pellet breaking speed) (Table 2). This problem is caused by the low density of the feed, thus it is easily destroyed in water before being consumed by the white shrimp *L.vannamei*. As reported by other researchers, the more optimal feed stability or water stability, the higher the organisms will utilize the feed and vice versa **(Saade *et al.*, 2011a; Safir *et al.*, 2022)**. Apart from that, it is thought to result from the unfermented soy flour content in the feed being too high, so it needs to be adequately digested. This is because soybean meals do not go through a fermentation process, hence the fiber content in the feed is high at 5.70% (Table 1). As reported by other researchers, feed containing high fiber is difficult to digest and inhibits the digestion process of other nutrients, resulting in low feed consumption levels **(Jannathulla *et al*., 2017b; Novriadi *et al*., 2023)**.

The efficiency of feed utilization is related to the increase in biomass weight in the organism's body, which comes from the use of protein in feed **(Nourhan *et al*., 2022; Hasnidar *et al*., 2024)**. The feed efficiency value is the result of a comparison between the increase in body weight of the Pacific white shrimp *L.vannamei* and the amount of feed consumed during the rearing period **(Omran *et al*., 2024)**. The research results showed that the highest feed utilization efficiency was recorded in the treatment with a substitution level of 50%, namely 81.44% (Table 2). High levels of consumption are one of the causes. This differs from the control treatment, which produces the lowest feed utilization efficiency compared to other treatments. The issue stems from inadequate feed digestion, as seen by the poor feed consumption rate of 81.44%. These results can be explained by the fact that fermented feed generally produces higher feed utilization efficiency values than feed without fermentation. It is suspected that there will be an increase in the number of microbes and enzyme activity that plays a role in nutrient metabolism in the digestive tract **(Klahan *et al*., 2023)**. In addition, the presence of mixed microbes in feed can simplify the molecular structure of feed, which is difficult to break down and stimulate the production of endogenous enzymes in the digestive tract, thereby increasing nutrient absorption for the Pacific white shrimp *L. vannamei* **(Hamsah *et al*., 2019)**. This problem has significant implications for feed digestibility and growth of *L. vannamei* **(Vidriales *et al*., 2021)**.

The research results shown in Table (2) show that treatment with a substitution level of 50% resulted in higher levels of body glycogen in the juvenile *L. vannamei* compared to other feed substitutes. This indicates that the balanced contribution of soybean flour and raintree seed meal produces protein levels in the feed according to the needs of *L. vannamei*. Therefore, the white shrimps have an optimal energy to support metabolic processes and growth. The increase in body glycogen is in line with the rise in absolute growth of *L. vannamei*; It is suspected that excess hemolymph glucose, once metabolic energy needs are met, is immediately converted into glycogen. This glycogen serves as an energy reserve that aquatic organisms can use when needed **(Zainuddin *et al*., 2019; Zhu *et al*., 2024)**. Hemolymph glucose, an essential energy source for *L. vannamei*, can also be formed from non-carbohydrate compounds such as protein and fat through gluconeogenesis **(Su *et al*., 2022)**. High blood glucose will be stored in the form of glycogen through glycogenesis. In contrast, if blood glucose levels are low, gluconeogenesis will convert glycogen back into glucose **(Manik & Arleston, 2021)**.

The lowest glycogen content was at the 0% treatment feed substitution level, which was also in line with the low absolute growth of the juvenile *L. vannamei*. This condition is caused by the absence of the addition of mixed microbes, which produce exogenous enzymes such as lipase, cellulase, glucoamylase, and invertase in the feed, hence it is thought to influence the low activity of digestive enzymes, with an impact on the low optimal utilization of feed nutrients **(Murueta *et al.*, 2017; Klahan *et al.*, 2023b)**. The absence of glucose deposits suggests that the energy provided is only sufficient for immediate metabolic needs, which impacts the low absolute growth of the white shrimp. Additionally, at a 0% substitution level, the crude fiber content is higher at 5.70% (Table 1). Crude fiber affects the energy value of the feed, as there is a correlation between fiber content and available energy: higher crude fiber reduces the energy available to shrimp since fiber components like cellulose, hemicellulose, and lignin are complex to digest and provide low energy **(Hemre *et al.*, 2002; Akinola *et al.*, 2022)**.

The highest absolute growth and survival of the juvenile *L. vannamei* (Table 2) were observed at a 50% substitution level with the fermented raintree seed meal microbial mixture. This result is attributed to the high total feed consumption and feed efficiency. These findings are supported by the high average body glycogen content of the juvenile *L. vannamei* in the 50% substitution feed.

The highest absolute growth of the juvenile *L. vannamei* (4.09g) observed in this study aligns with the finding of **Wang *et al.* (2020)**, who reported that substituting soybean meal with fermented cottonseed meal and iron enrichment improved digestive enzyme activity, non-specific immunity, phenoloxidase activity, and growth in the juvenile *L. vannamei*. **Han *et al.* (2022)** also found that substituting soybean meal with fermented cottonseed meal up to 75% positively impacted growth performance, antioxidant capacity, and essential amino acid content in the white shrimp compared to controls. In contrast, using unfermented soybean meal at 0% substitution with fermented raintree seed meal resulted in poor growth due to low feed dispersion and density, which hindered optimal feed utilization **(Ashry *et al.*, 2023)**. Furthermore, unfermented soybean meal can cause an imbalance in amino acids and fatty acids, potentially leading to an intestinal inflammation in *L. vannamei* **(Peng *et al.*, 2023)**.

The substitution of soybean meal with fermented raintree seed meal mixed with microorganisms resulted in varying survival rates (SR) for the white shrimp *L. vannamei* across different studies. A 50% substitution level resulted in the highest survival rate compared to other treatments. However, survival rates decreased with 25, 75, and 100% substitutions and were the lowest in the control treatment. This may be due to the cannibalistic behavior of *L. vannamei* post-moulting. The 50% fermented raintree seed meal substitution improved shell-hardening speed post-moulting, reducing predation among shrimp. Adequate nutrition supports faster metabolic processes, as evidenced by high feed consumption and body glycogen levels. In contrast, the control treatment, with longer shell-hardening periods, led to an increased cannibalism due to slower post-moulting recovery and lower nutritional adequacy. Previous research has also postulated that high cannibalism rates can negatively impact the survival rate of *L. vannamei* due to inadequate nutrition during rearing **(Shao *et al.*, 2020; Rivaie *et al.*, 2023)**.

CONCLUSION

The FTIR analysis showed a change in hydrogen bonds to amine groups and a strengthening of the group function in raintree seed meal, which was fermented using mixed microbes. Furthermore, the results of *in vivo* research concluded that fermented raintree seed meal with a mixture of microorganisms could replace 50% of soybean meal in feed, without negatively affecting the growth performance of the juvenile *L. vannamei*.

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